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STUDIES ON DEFECATION, WITH SPECIAL REFERENCE TO A MEDULLARY DEFECATION CENTER*

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HATCHER and Weiss (1923) in studying the effects of direct application of drugs to the vomiting center found that small doses of picrotoxin, heroine, and several other drugs produced in many instances prompt evacuation of the bowels. They postulated, therefore, a medullary center for the reflex act of defecation, which must lie in close proximity to the vomiting center. The present investigation was undertaken to study in greater detail the nature of this center.

I. THE DEFECATION REFLEX

In the strict sense of the word *defecation* or voiding feces from the body refers only to the actual expulsion of fecal matter through the anus, while the term *straining* indicates the assumption of the characteristic defecation posture accompanied by contractions of the abdominal muscles, aiding expulsion. It is obvious that the simultaneous occurrence of so many coordinated movements in normal as well as in decerebrated animals must be mediated by a complex reflex mechanism.

When the fecal matter leaves the sigmoid and enters the rectum, it produces a sense of fullness and elicits a call to defecation. The distention of the rectum sets up afferent impulses, traveling probably along the lower lumbar and sacral sensory nerves to a center or centers. The efferent im-

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pulses from these centers, passing by way of the lumbar sympathetics and sacral parasympathetics, produce peristalsis of the large intestine, especially the rectum, and relaxation of the sphincters; passing along the spinal nerves, they produce contractions of the abdominal musculature. The dog thereupon assumes a typical posture, enabling it to force the fecal matter out of the rectum. The pelvis is lowered several inches below its normal position by flexion of the hind legs, more marked at the knee than at the hip joint, the legs are but slightly separated and almost parallel, the tail is abducted upward, and the external sphincter contracts and relaxes alternately. The reflex character of all these movements is demonstrated by the fact that they also occur in decerebrated animals.

Defecation posture, however, may be elicited by afferent impulses originating from points other than the rectum. We removed the entire large intestine, established an ileal fistula, and allowed the dogs to recover from the anesthesia. Such dogs frequently assume the defecation posture while expelling liquid, or at the later stages of the experiment, semisolid material through the fistulous opening. Oldberg and Walsh (1928) also observed several ileostomized dogs "in the act of attempted defecation" when they had only an inch or so of rectum intact.

II. THE LOCALIZATION OF THE DEFECATION CENTER

a. *Methods of Study: The Choice of Evacuants and Emetics.*—If there is a center for reflex defecation, its presence can be demonstrated in two ways, by stimulating the center with a drug, causing defecation; and by the failure of that drug to cause defecation after the center has been excluded. This method of experimentation calls for the use of a purgative that has been definitely shown to be central in its action. The work of Hatcher and Weiss suggested the use of picrotoxin or heroine. Picrotoxin, although producing prompt defecation when applied directly to the floor of the fourth ventricle, is rather uncertain in its action when administered systemically. A mixture of picrotoxin and heroine hydrochloride was then tried on the assumption that the latter drug might act synergistically with the former. This mixture (picrotoxin, 0.5 mg., heroine hydrochloride, 0.4 mg.)* injected intramuscularly produced defecation in from two to twenty-one minutes in eight dogs. Intravenous injections of such mixtures (picrotoxin, 0.06 mg., heroine hydrochloride, 0.12 mg.) produced defecation in from one to five minutes in thirteen out of fourteen dogs. Codeine sulphate alone gave equally satisfactory results, hence subsequent experiments were carried out using only codeine sulphate. An intravenous dose of 0.8 mg. of codeine sulphate produced defecation in less than three minutes in fourteen dogs; in about one-half hour in two dogs; while in two dogs the results were negative. In cats the minimum evacuant dose of codeine is much higher, 8 mg. producing defecation in ten out of thirteen cats.

A series of experiments were carried out to determine the seat of action of codeine. Codeine sulphate injections were found ineffective following

*All doses are expressed in terms of milligrams of the drug per kilogram of body weight. To avoid repetition, the words "per kilogram of body weight" are omitted.

double vagotomy in four dogs; whereas pilocarpine hydrochloride in the same dogs produced defecation, suggesting that codeine acts centrally or peripherally on afferent structures as a purgative. The protocol of a typical experiment of this series follows.

January 30, 1930.	Male dog, weight 12.7 kg.	
	10:05 A.M.	Both vagi cut in neck.
February 1, 1930.		
	10:31 A.M.	2 mg. codeine sulphate intravenously.
	10:35 A.M.	Drowsiness.
	10:31-11:20 A.M.	No straining or defecation.
	11:20 A.M.	1 mg. pilocarpine hydrochloride intravenously.
	11:30 A.M.	Violent straining × 2.
	11:31 A.M.	Straining, defecation × 2.
	11:33 A.M.	Urination.

A direct proof that codeine may act centrally as a purgative was afforded by the experiments on the localization of the defecation center, in which codeine failed to produce purgation after the exclusion of a definite area in the central nervous system.

Besides codeine, a centrally-acting evacuant, we used a typical peripherally-acting purgative, pilocarpine, the contrast in the effects produced serving to control the codeine experiments.

In attempting to exclude the defecation center by ablation or by drug paralysis, it was necessary to determine the success of the exclusion by testing the functional presence or absence of a better-known center in the same area, i.e., the vomiting center as it was thought that measures which destroyed one would destroy the other. For this purpose we used the typical centrally-acting emetic apomorphine, and a number of other emetics, such as pilocarpine, digitalis, antimony and potassium tartrate (administered systemically); copper sulphate, zinc sulphate, mercuric chloride and antimony and potassium tartrate (administered orally).

The concept that codeine acts centrally as a purgative is in agreement with the accepted theory of the action of opiates, which are known to affect the medullary centers. It is not feasible to review here the entire literature on the effect of opiates on the gastrointestinal tract especially since most authors concerned themselves with the explanation of the constipating rather than the purgative action of these drugs.

Magnus (1903) believed that the purgation effected by opiates is due to their action on the intestinal wall, producing increased motility. Pal (1900) stated that opium and morphine stimulate the ganglia in the wall of the small intestine, and that intravenous morphine injections cause immediate, active contractions involving both the long and the circular muscles, the rectum behaving similarly. Pal evidently did not believe that the action is central, although he admitted the fact that certain opium constituents increase the reflex excitability of the cord. Dixon (1903) stated that among the opiates the greatest purgative effect is obtained with apocodeine, less with apomorphine, still less with codeine, and least with morphine. He pointed out that the seat of apocodeine action cannot be upon the brain or medulla, since trans-

section of the cord and double vagotomy does not abolish its purgative action. He assumed that apocodeine purgation is due to the depression of the inhibitory sympathetic ganglia. In contrast to this we have shown that double vagotomy alone abolishes the purgative action of codeine. It should also be noted that codeine was found to be a more efficient purgative than Dixon would have us believe. Perhaps the preparation or the doses of the drug used were different.

b. *The Effects of Experimental Lesions in the Floor of the Fourth Ventricle.*—The observations of Hatcher and Weiss made it plain that the medullary defecation center lies in the vicinity of the vomiting center. Following their method of localizing the vomiting center, we performed a number of experiments in which we ablated some of the structures in the floor of the fourth ventricle, and tested the extent of the destruction by administration of emetics and centrally-acting evacuants. In two dogs which were allowed to survive the ablation for several months, the extent of injury was determined by microscopic examination.

1. Acute Experiments: In six dogs under ether anesthesia the floor of the fourth ventricle was exposed, and the destruction of the nuclei of the ala cinerea and its vicinity was attempted with a dull needle. The wound was then dressed, the animal was released and allowed to recover from the anesthesia. In about four hours, after the animal was able to move about normally, the destruction of the region of the vomiting center was tested by the intramuscular injection of 0.5 mg. of apomorphine hydrochloride. Two animals out of six retched and vomited repeatedly within a few minutes after injection, whereas in the other four, apomorphine failed to produce vomiting. To these latter animals which thus showed functional signs of destruction in the floor of the fourth ventricle, centrally-acting evacuants were administered. A mixture of 0.5 mg. picrotoxin and 0.4 mg. heroine hydrochloride and another mixture of 0.4 mg. picrotoxin and 4 mg. codeine sulphate failed to produce salivation, straining or defecation in two dogs. In the remaining two animals, the intravenous injection of 1 mg. codeine sulphate was equally ineffective. In three of the four dogs which failed to vomit following apomorphine, or to defecate following codeine injection, peripherally-acting emetics such as zinc sulphate or copper sulphate were administered by stomach tube. All three animals showed nausea, retching and vomiting, usually about twenty minutes after administration of a moderate dose. The following protocol is representative of the series.

December 7, 1929. Female dog, weight 6.0 kg.

9:08-10:30 A.M.	Ether anesthesia. Floor of the fourth ventricle exposed; large section of brain tissue destroyed in the lower triangle of the rhomboid fossa. Wound dressed; animal released.
2:13 P.M.	Animal walking about; condition good.
2:14 P.M.	0.4 mg. picrotoxin and 4 mg. codeine sulphate intramuscularly.
2:14-3:13 P.M.	No salivation, straining or defecation.
3:13 P.M.	0.5 mg. apomorphine hydrochloride intramuscularly.
3:16-3:28 P.M.	Depression.
3:13-4:30 P.M.	No nausea, retching or vomiting.

4:30 P.M.	50 mg. zinc sulphate (5 per cent solution) by stomach tube.
4:39 P.M.	Nausea.
4:50-4:53 P.M.	Retching, vomiting × 4.
5:00 P.M.	Dog killed.

Autopsy shows a probable destruction of the ala cinerea and also some of the deeper structures in the floor.

2. Chronic Experiments: In two etherized dogs the floor of the fourth ventricle was exposed with aseptic precautions, and a large section of tissue was removed in the lower triangle of the rhomboid fossa. The wound was closed and dressed, and the animal allowed to recover. One of the dogs was killed one hundred and forty-four days, the other ninety-six days, after the operation.

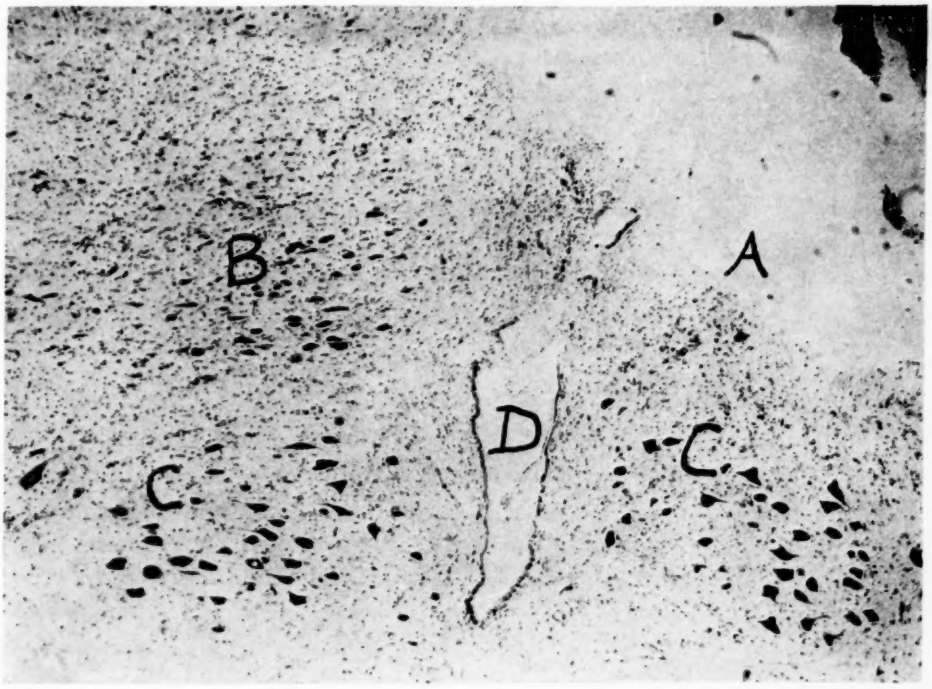

Fig. 1.—Dog A. Section through the caudal end of the hind-brain, showing complete destruction of the dorsal vagus nucleus and the nucleus of the solitary tract on the right side. *A*, Region of missing right nucleus. *B*, Left dorsal vagus nucleus. *C*, Hypoglossal nuclei. *D*, Central canal.

Dog A., male, 8.6 kg., a few hours after operation, failed to show either vomiting following apomorphine or defecation following picrotoxin-heroine injections. This condition persisted for four days. It was noticed, however, six days after operation that the animal retched following apomorphine injection. From the sixth to about the twentieth day, apomorphine caused vomiting, and a mixture of picrotoxin and heroine caused defecation in the dog. Within less than a month, however, the animal lost its ability to respond to these centrally-acting drugs. This condition of failure to react to these drugs was maintained until the dog was destroyed one hundred and forty

days after operation, its reaction to these drugs being tested on twelve occasions during this period. At the same time the dog also failed to vomit after the intravenous injection of 1 mg. of pilocarpine hydrochloride, 0.4 cat units of tincture of digitalis, and 50 mg. of antimony and potassium tartrate. Nevertheless, it showed typical nausea, retching and vomiting after oral administration of 50 mg. of zinc sulphate, and after 100 c.c. of a 1 per cent solution of copper sulphate (total).

At the conclusion of the experiment the hind-brain of the dog was removed and fixed in 10 per cent formalin solution, embedded in paraffin and sectioned. The sections were stained for fifteen minutes in cresylecht violet

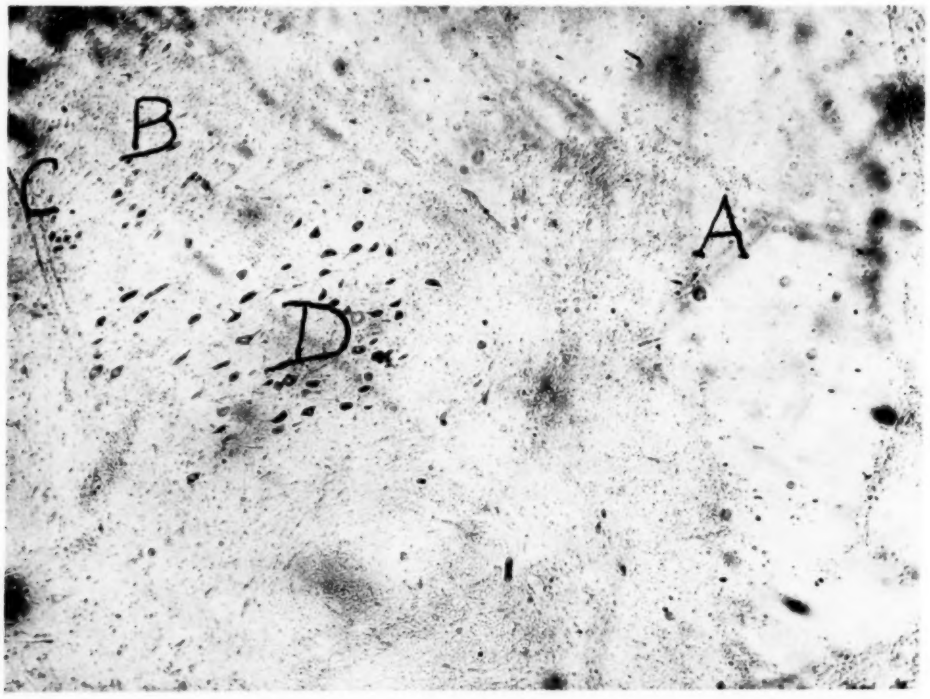

Fig. 2.—Dog A. Section through the mid-region of the hind-brain, showing complete destruction of the dorsal vagus nucleus, the nucleus of the solitary tract and hypoglossal nucleus on the right side. On the left side parts of the dorsal nucleus of the vagus and the nucleus of the solitary tract are still present, but all the cells in these nuclei show degenerative changes. The left hypoglossal nucleus is intact and has a normal appearance. *A*, Region of the missing right nuclei. *B*, Left dorsal vagus nucleus. *C*, Nucleus of the left solitary tract. *D*, Left hypoglossal nucleus.

and mounted. The systematic examination of the slides showed that the extreme caudal end of all the nuclei located in the floor of the fourth ventricle were intact, and that damage to the nuclei was more and more pronounced as one proceeded cephalad. At the lower level, the dorsal and the sensory nuclei of the vagus showed extensive destruction only on the right side; somewhat further cephalad the damage of the left nuclei was also noticeable; while still further both visceromotor and sensory (nucleus of the solitary tract) vagal nuclei, as well as the hypoglossal nuclei, were destroyed. Some

of the cells of the nuclei that were present showed distinct degenerative changes, especially in the cell nuclei and the Nissl bodies. (See Figs. 1, 2, and 3.)

Dog B, female, 7.5 kg., was operated upon in the same manner as Dog A. On the following day, it failed to respond to apomorphine or codeine. It responded frequently, however, to these drugs from the eighth to the forty-seventh day. After this period, apomorphine failed to cause vomiting, and

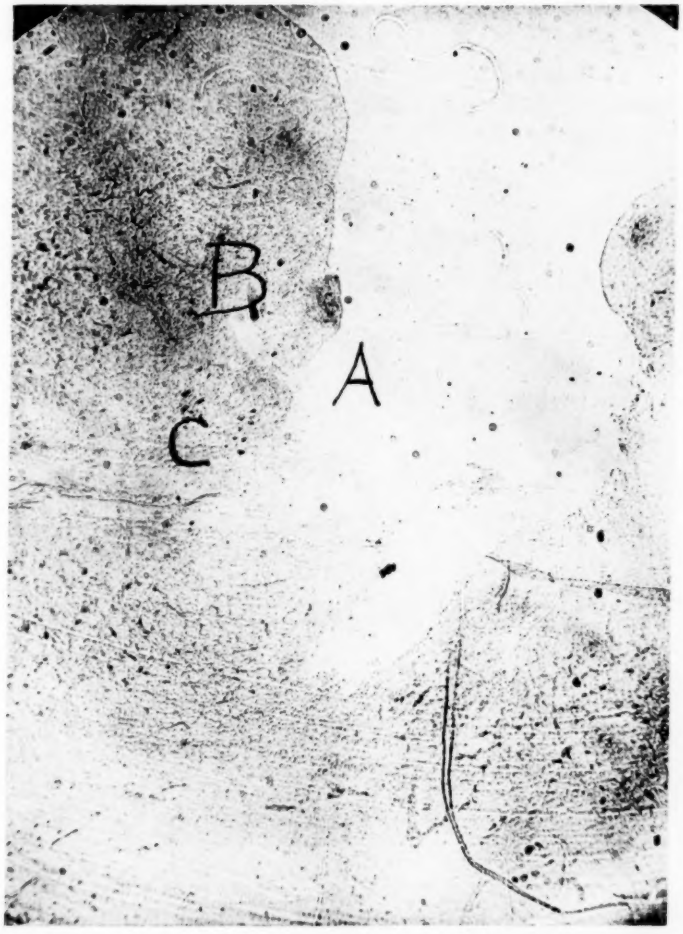

Fig. 3.—Dog A. Section through the cephalad end of the hind-brain showing complete destruction of the dorsal vagus nuclei and the nuclei of the solitary tract on both sides. The right hypoglossal nucleus is completely, the left only partially, destroyed. *A*, Region of the missing right nuclei. *B*, Region of the missing left nuclei. *C*, Left hypoglossal nucleus.

codeine failed to produce defecation. It may be emphasized that in this animal, even when apomorphine provoked violent vomiting, there was a failure to respond to 0.4 cat units of tincture of digitalis, whereas eight normal dogs showed prompt retching and vomiting after the same dose of this preparation of digitalis. The animal, while not responding to systemically-administered emetics, vomited after the oral administration of moderate doses of zinc sulphate, copper sulphate and antimony and potassium tartrate.

On the ninety-sixth day of the experiment the animal was killed, and the hind-brain was removed and fixed in 10 per cent formalin solution. After being treated like the hind-brain of Dog A, the sections showed the following changes in the floor of the fourth ventricle: At the caudal end all nuclei were intact, while further cephalad the dorsal nucleus of the vagus showed degeneration. Still more anteriorly, the degeneration extended over to the left side. At the extreme cephalic end all nuclei again appeared normal. While the nuclei of the solitary tract showed no damage on either side, it is pretty difficult to say whether or not they were in a normal state. In this dog, as in Dog A, many of the cells, though present, showed degenerative changes of the cell nuclei and the Nissl bodies. (See Figs. 4 and 5.)

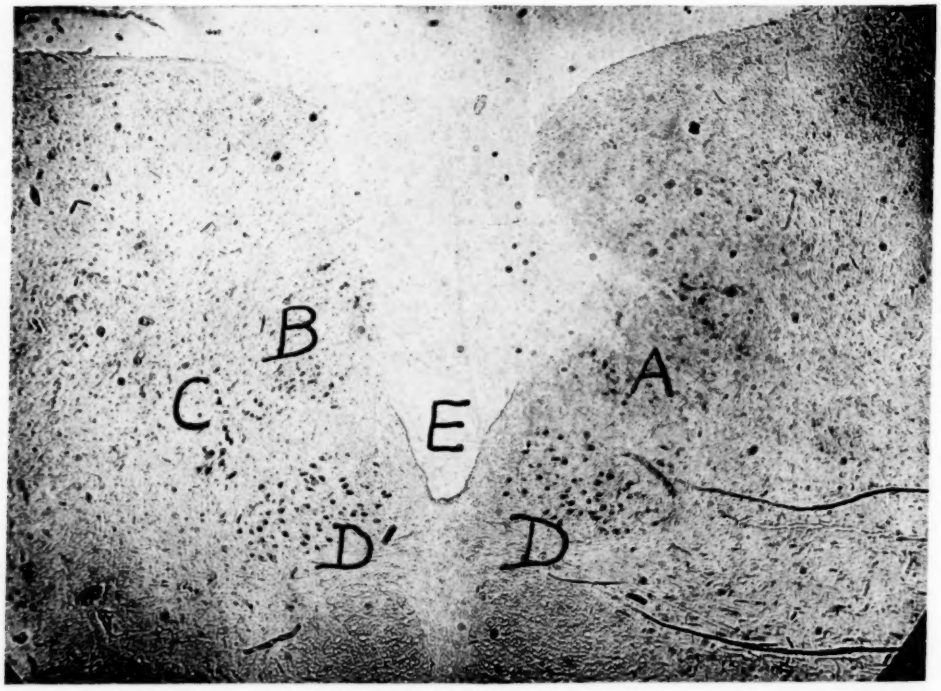

Fig. 4.—Dog B. Section through the caudal end of the hind-brain, showing partial destruction of the right dorsal vagus nucleus and the nucleus of the right solitary tract, the remaining cells showing degenerative changes. Some of the cells of the left dorsal vagus nucleus also show degeneration. Both hypoglossal nuclei are intact. *A*, Remnants of the right dorsal vagus nucleus. *B*, Left dorsal vagus nucleus. *C*, Nucleus of the left solitary tract. *D*, Hypoglossal nuclei. *E*, Central canal.

The results of both acute and chronic ablation experiments seem to establish the fact that it is possible to abolish defecation from picrotoxin-heroine or codeine by removing a part of the brain tissue in the floor of the fourth ventricle. We are therefore forced to conclude that there is a center in this region which is affected by codeine, perhaps very much in the same way as the vomiting center is affected by apomorphine. The histologic studies of the brains furnished evidence supporting the concept that both centers may be looked for in the region of the visceromotor and sensory vagal nuclei.

Since injections fail to produce defecation even in the presence of parts of the dorsal and sensory vagal nuclei, it is possible that not all the cells located in this region are concerned in the reflex act of defecation. An exact localization of the center is so far not possible.

The experiments also furnished further data on the mechanism of vomiting. They made clear the fact that animals which do not vomit from systemically-administered emetics may still vomit from orally-administered emetics. It is exceedingly difficult to evaluate these results. Two alternative provisional interpretations may be offered.

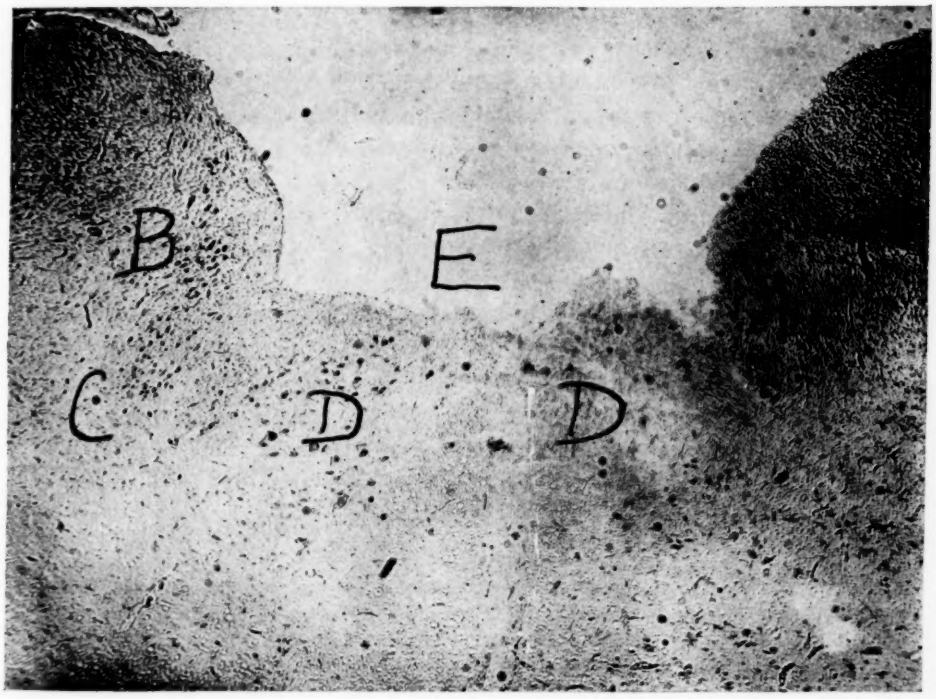

Fig. 5.—Dog B. Section through the middle part of the hind-brain, showing approximately the same condition of the vagus nuclei as Fig. 4. The right hypoglossal nucleus is completely, the left partially, destroyed. *A*, Remnants of the right dorsal vagus nucleus. *B*, Left dorsal vagus nucleus. *C*, Nucleus of the left solitary tract. *D*, Hypoglossal nuclei. *E*, Fourth ventricle.

1. In the ablation experiments, as well as in the experiments where morphine is applied to the floor of the fourth ventricle, the vomiting center is not completely excluded, so that there are enough cells and paths left to be reached by such powerful afferent stimuli as are set up by peripherally-acting emetics, such as zinc and copper sulphate.

2. There may be a complete reflex arc for vomiting below the medullary center and independent of it or a center above it which is brought into play only under special circumstances.

There are several phenomena brought to light in the above experiments that are difficult to explain. The dogs which survived the destruction of the

vomiting center over a considerable period showed a temporary recovery of the vomiting center as judged by the response to apomorphine. We may provisionally explain the recovery by assuming that immediately after the operation vascular changes occur with swelling and compression of the nerve cells and paths. The recovery of function may be due to the subsidence of the vascular reaction, while the permanent failure to vomit may be explained by the secondary degeneration of the structures, which results in a permanent loss of function.

It is exceedingly difficult to determine the exact location of the vomiting center. Hatcher and Weiss stated that the center may be in the sensory nuclei of the vagi, but it is almost certain that the nuclei of the ala cinerea, and the fibers going from the nuclei of the solitary tract to the visceromotor nuclei and other motor centers, are also necessary for typical vomiting. The main conclusion we can draw from the ablation experiments is the necessity of working with animals surviving the operation for some time because only in such animals can one get a clear picture of the actual and permanent damage done to the nerve centers in the medulla.

c. *The Paralysis of the Defecation Center by Local Application of Morphine Sulphate.*—The intravenous injection of morphine sulphate cannot be utilized for the exclusion of the defecation center since the animals frequently show violent and persistent diarrhea shortly after its administration. After morphine was injected intravenously, apomorphine failed to produce emesis; while under the same conditions the oral administration of mercuric chloride was followed by vomiting. The protocol of an experiment of this type is given.

October 25, 1929. Female dog, 4.5 kg.

9:50 A.M.	5 mg. morphine sulphate intravenously.
12:40 P.M.	0.25 mg. apomorphine hydrochloride intramuscularly; animal sitting up.
12:40-1:20 P.M.	No nausea, retching or vomiting.
1:22 P.M.	25 mg. mercuric chloride (1:1000) by stomach tube.
1:27 P.M.	Regurgitation.
1:32-1:33 P.M.	Typical retching and vomiting × 2.

Because of the impracticability of administering morphine intravenously in the above connection, the drug was applied directly to the floor of the fourth ventricle. In five dogs 0.05 c.c. (total) of a 4 per cent solution of morphine sulphate was painted over the vomiting center and its vicinity with a camel's-hair brush. In all the five dogs this procedure abolished defecation after codeine administration, while it failed to abolish defecation after pilocarpine administration. Table I shows the results of these experiments. The fact that the animals did not vomit after 1 mg. of pilocarpine hydrochloride indicates that the vomiting center was paralyzed with respect to this drug at the same time that the defecation center was paralyzed with respect to codeine. The animals, although not responding to apomorphine or pilocarpine injection, vomited after the oral administration of strong local irritants, as zinc sulphate. The following is the protocol of a typical experiment.

November 19, 1929. Female dog, 4.2 kg.

10:35-11:00 A.M.	Floor of the fourth ventricle exposed under ether anesthesia.
11:10 A.M.	0.05 c.c. (total) morphine sulphate painted on the floor.
12:15 P.M.	1 mg. pilocarpine hydrochloride intravenously.
12:15-2:00 P.M.	No vomiting.

November 21, 1929.

1:20 P.M.	0.25 mg. apomorphine intramuscularly.
1:20-2:06 P.M.	No vomiting.
2:06 P.M.	100 mg. zinc sulphate (5 per cent solution) by stomach tube.
2:10-2:20 P.M.	Retching, vomiting × 7.

The experiments described above show conclusively that defecation of central origin may be prevented by paralysis of certain structures in the rhomboid fossa. The results parallel in two respects those obtained by ablation of these structures—1. Codeine defecation is abolished. 2. Vomiting from apomorphine and pilocarpine injection is abolished, but vomiting from the administration of local irritants is preserved.

TABLE I

SHOWING THE EFFECT OF CENTRALLY AND PERIPHERALLY-ACTING EVACUANTS AFTER THE APPLICATION OF 0.05 C.C. OF 4 PER CENT MORPHINE SULPHATE SOLUTION TO THE FLOOR OF THE FOURTH VENTRICLE

NO.	WT. OF ANIMAL (KG.)	TIME OF APPLICATION OF MORPHINE SULPHATE TO THE CENTER	TIME AND DOSE OF INTRAVENOUS INJECTION OF CODEINE SULPHATE	RESULT	TIME AND DOSE OF INTRAVENOUS INJECTION OF PILOCARPINE HYDROCHLORIDE*	TIME ELAPSED BETWEEN INJECTION OF PILOCARPINE HYDROCHLORIDE AND DEFECATION (MINUTES)
1	4.7	9:50 A.M.	10:50 A.M. (2 mg.)	Negative	11:25 A.M. (1 mg.)	4.5
2	6.6	10:10 A.M.	11:06 A.M. (1.5 mg.)	Negative	11:36 A.M. (1 mg.)	5
3	5.8	10:03 A.M.	11:10 A.M. (2 mg.)	Negative	11:40 A.M. (1 mg.)	1
4	9.4	11:20 A.M.	12:48 P.M. (2 mg.)	Negative	1:25 P.M. (1 mg.)	2
5	4.2	10:10 A.M.	11:05 A.M. 1 mg.)	Negative	12:15 P.M. (1 mg.)	1

*NOTE. There was no vomiting in any of the animals after the pilocarpine injections. The duration of the depression of the center by morphine was studied in dogs 4 and 5. In both dogs codeine and apomorphine were inactive on the second day, and active only on the third. Dog 5, while not responding to apomorphine, did vomit following the oral administration of zinc sulphate.

d. *The Action of Drugs Applied to the Defecation Center.*—Following the method of Thumas (1891) and of Hatcher and Weiss (1923), drugs were applied to the floor of the fourth ventricle, and the effects observed. In four experiments these drugs (picrotoxin, heroine, and codeine) failed to produce defecation, while in four additional experiments defecation took place seven, eleven, nineteen, and twenty minutes following the application of the drugs to the floor of the fourth ventricle. In two experiments on one dog we obtained defecation five minutes after the application of a mixture of 0.0005 mg. of picrotoxin and 0.001 mg. of heroine hydrochloride; and three minutes fol-

lowing the application of 0.00025 mg. of picrotoxin and 0.0005 mg. of heroine hydrochloride. In two dogs the application of 0.03-0.06 mg. of codeine sulphate to the center produced diarrhea in two and six minutes respectively. In one experiment the application of 0.05 c.c. of a 4 per cent solution of morphine sulphate produced defecation in two minutes.

These experiments, together with those of Hatcher and Weiss, seem definitely to establish the existence of a defecation center in the vicinity of the vomiting center.

III. THE EFFERENT AND AFFERENT PATHS OF THE DEFECATION REFLEX

The following experiments are only preliminary, and need further elaboration and elucidation.

a. *Efferent Paths.*—In four dogs anesthetized with ether both vagi were cut in the neck. During the days following, the intravenous injection of 1 to 2 mg. of codeine sulphate failed to cause defecation, while the injection of 1 mg. of pilocarpine hydrochloride invariably resulted in defecation. Furthermore, the intravenous injection of 10 mg. of atropine sulphate in three intact dogs was also effective in preventing codeine defecation.

In the light of the above experiments, it seems that the efferent arm of the medullary defecation reflex is mainly parasympathetic. It should be noted, however, that doubly-vagotomized dogs are not only able to empty the bowels, but also assume the typical defecation posture after pilocarpine injection, and under other conditions.

b. *Afferent Paths.*—Merzbacher (1902) found that the section of the sensory roots of the last lumbar and of the sacral nerves abolished all sensation from the rectum and anus, so that the dogs never assumed the typical defecation posture. In seeming contradiction to these experiments, we found that typical defecation posture may be observed in dogs with the entire large intestine removed. Codeine injection produces in such ileostomized dogs not only defecation from the ileal fistula but a typical straining accompanying this act. The following protocol is characteristic of the results obtained in three dogs.

January 22, 1930. Male dog, weight 4.5 kg.	
9:50-10:40 A.M.	Ileostomy performed under ether anesthesia.
January 28, 1930.	
9:40 A.M.	Dog in good condition.
9:43 A.M.	1 mg. codeine sulphate intravenously.
9:44 A.M.	Nausea.
9:45 A.M.	Defecation posture. Typical straining; powerful contraction of the abdominal muscles; expulsion of fluid from the fistulous opening.
9:48 A.M.	Strained again without expelling anything.

Thus afferent impulses reaching the medullary defecation center may originate from the small intestine. If the defecation center is of similar reflex nature to the vomiting center, then codeine may be said to act in this type of an experiment simply by lowering the threshold of the center for stimuli coming from the small intestine.

It is important to state that Goltz and Ewald (1896) saw defecation and the assumption of the typical posture not only in decerebrated animals but also in dogs with high dorsal or low cervical transsection of the cord. Since both spinal and vagotomized dogs defecate, it is obvious that defecation can be brought about without the medullary center. As a matter of fact, there seems to be an anospinal center in the terminal conus which is connected with the anal sphincters by means of the lumbar sympathetic and the sacral parasympathetic. Many observations indicate that there is an even lower center for defecation in the cells of the peripheral nerve plexuses—the source of the nerve supply to the plain musculature of the rectum and the internal sphincter. After the exclusion of the terminal conus, the animals regain within a short period the capacity to defecate. It is also well known that the external sphincter of the anus is subject to will, and Bechterew (1893) succeeded in isolating its cortical center behind the cruciate sulcus.

To apportion the rôle played by the respective centers in the normal act of defecation, one must first consider that the cortical center is not directly concerned with the defecation reflex but simply prevents the emptying of the rectum by a "voluntary" act of the animal. It is difficult to determine the exact part played by the medullary and the spinal center in defecation. Goltz and Ewald's dogs, and also the dogs with the medullary center excluded by ablation or by morphinization, are capable of defecating. This indicates that the medullary center is not always indispensable in the defecation act. When a centrally-acting purgative, such as codeine, is administered, however, or in any instance where the defecation impulse starts from the small intestine and is therefore not under the control of the terminal conus, the defecation may be due to stimulation of the medullary center. Fright, which seems to affect several medullary centers, also frequently produces purgation. This indicates the possibility that the medullary center for defecation has mainly an emergency function, and is superimposed over the primitive anospinal center. Such a conception of the medullary center for defecation is in agreement with the facts that indicate its close connection with the vomiting center. The vomiting act, one may say, certainly serves emergency purposes, while everyday defecation does not. Should the emergency arise, however, necessitating a thorough emptying of the gastrointestinal tract, the purgation would probably be mediated by the medullary vomiting center, if the irritant substance were in the upper gut, and by the medullary defecation center if it were in the lower gut. These two centers are well differentiated, in the dog at any rate, some drugs affecting each of them selectively. It should be remembered, however, that apomorphine frequently produces defecation, and that codeine almost invariably produces nausea before evacuation. This may suggest that these centers are acted upon by essentially the same kind of stimuli, and that their ultimate response depends largely upon the state of the peripheral organs.

IV. SUMMARY

1. Codeine, picrotoxin and heroine are centrally-acting evacuants.

2. The application of centrally-acting evacuants to the floor of the fourth ventricle may elicit straining and defecation.

3. Lesions in the floor of the fourth ventricle, or application of morphine sulphate to the floor, may abolish the straining and defecation which follow the injection of centrally-acting evacuants.

4. Afferent impulses for defecation may originate in the small intestine.

5. The vagus nerve carries efferent impulses concerned in defecation of medullary origin.

6. Dogs that did not vomit from apomorphine and did not defecate following codeine administration failed to vomit following the *injection* of pilocarpine, digitalis and tartar emetic, but vomited upon *oral* administration of local irritant emetics, tartar emetic, zinc sulphate and mercuric chloride.

7. The nature of the vomiting and defecation centers is discussed in the light of the experimental data obtained.

The author is under deep obligation to Dr. Robert A. Hatcher for suggesting the problem and for his constant interest during the course of the investigation. The generous cooperation of Dr. Philip B. Armstrong, who carried out the histologic studies concerned with the localization of the centers and of Dr. William P. MacNamara, who performed the ileostomies made this work possible. I also wish to thank Dr. Josuah Sweet, who kindly permitted us to use the facilities of his laboratory.

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ANTIMONY IN MEDICINE

By C. N. Myers, Ph.D., and Binford Throne, M.D., Brooklyn, N. Y.

OF ALL the medicaments associated with metals antimony stands in a position unique in that it has been enthusiastically praised and violently attacked. The fashionable women of antiquity used it for painting their eyebrows. Mention is made of the use of the black sulphide by Queen Jezebel in the so-called fourth book of Kings in which it was said "But Jezebel hearing of his coming painted her face with stibic stone." Hippocrates and other Greek writers recommended it as a collyrium for the eyes, the eye salve mentioned in Revelations iii, 18th verse ". . . . and anointest thy eyes with eye salve that thou mayest see." All through the Middle Ages antimony was used as an astringent to the eyes and as a caustic in cases of cancer, hemorrhoids, and open wounds.

In about 1600 the Currus Triumphalis Antimonii, fictitiously ascribed to the monk Basil Valentinus, extolled the metal as a universal remedy. A long and bitter controversy arose, but although the use of antimony was banned by the official pharmacists, it came to be employed largely for various diseases in the two succeeding centuries. Valentinus recommended the mixture of antimony, lead, and mercury in the treatment of syphilis. The name "Antimony," Anti-Moine, is attributed to him. During the course of his observations it was found that antimony would fatten pigs but when administered to lean and poorly nourished friars the effect was fatal. To be exact, antimony is good for pigs but bad for monks.

The controversy waged so violently that the Faculty of Physicians of Paris forbade its use in 1566. About forty years later it expelled one of its most able associates, Turquet de Mayerne, who migrated to England and became Sir Thomas Mayerne, a favorite physician to the king and one of the editors of the first London Pharmacopoeia in 1618.

In 1657 Louis XIV was taken ill at Calais and was cured of his alleged typhoid fever with Tartar Emetic. This marked the defeat of the antiantimonists and in 1666, just 100 years after its promulgation, a repeal of the decree was issued.

Several salts, especially Kermes Mineral, or red sulphide, and diaphoretic or calcined antimony were used as eliminants, as purgatives, and for epilepsy and hectic fevers. The famous James's powder consisted for the most part of antimony oxide. Pulvis Jacobi Verus used in febrile cases was an imitation of James's powder. Fothergill's pill was antimony combined with aloes, scammony and colocynth. Plummer, Professor of Chemistry at Edinburgh (1730-1740) prepared a pill consisting of calomel, antimony sulphide and guaiacum. This was largely used in gouty conditions. Dr. Archibald Billing (1822-45) said that antimony was one of the most useful of all medicines.

His clinical records present a most convincing aspect applicable even to our present day, namely, that clinicians develop skill in handling drugs and get more out of them than those who use the same preparation intermittently. At one period in the development of antimony therapy the drug fell into the class of so-called alteratives which are alleged to produce change in organic textures, and consequently alteration of function.

The theory of alterative action took a firm hold of the medical mind. It was a cloak for ignorance, no doubt; yet there may have been, in the case of antimony and other drugs, empirical facts which underlie the belief. With the growth of scientific therapeutics these ill-understood remedies tended to be discarded, and gradually antimony has fallen into the background and has been forgotten, save for the occasional prescription of the wine in catarrhs. The drug has come to be left, in London at any rate, to the veterinary surgeons, who give it to horses to improve their coats. Plummer's pill is very seldom prescribed, at any rate in London, but I am told that it is sometimes administered in chronic iridocyclitis, the origin of which is suspected to be of a syphilitic nature. The late Sir J. Hutchinson used to give small doses of the wine in some skin affections, and it has lately been recommended by Mr. Willmott Evans in dermatitis herpetiformis; he gives doses of from 3 to 10 minims, and it has, he says, a marked influence on the disease, especially in allaying itching. It is also given in lichen planus.

Modern chemistry, which has shown that antimony is a triad element, ranging with nitrogen, arsenic, and bismuth, has led to its trial in some disorders which are more or less amenable to arsenic. The most striking instance is in the case of certain diseases due to the presence in the blood of animal organisms belonging to the class of protozoa, or allied therewith. It was found that the trypanosoma, the parasite of sleeping sickness, is influenced by arsenic. Seeking to obtain a more effectual agent for the destruction of this parasite, Prof. Cushny was led in 1907 to try antimony. He used at first a compound of the metal with glycine, and later tartar emetic or its sodium analogue, injecting the remedy into rats affected with the disease, with the result that the parasites were destroyed, and in 23 cases out of 25 there was no recurrence. A like success was soon attained in the human subject. It was found advisable to give both arsenic and antimony, in alternation, thus avoiding any irritant or caustic effects. This treatment has now been used in many instances, and the dread disease of sleeping sickness, the scourge of some parts of Africa, has thus been brought under control.

The value of antimony in acute disease, once so much esteemed, may prove to be chiefly limited to disorders in which certain parasites are present. Yet the practice of giving minute doses of tartar emetic in sthenic pulmonary and other fevers until vomiting is produced is worthy of remembrance. Emesis is seldom now purposely resorted to, yet every medical man meets with instances in which an act of copious vomiting, spontaneously or accidentally induced, has relieved a patient. This relief may come from the emptying of clogged bronchial tubes which accompanies vomiting, or the actual clearance of the stomach and duodenum may leave the chylopoietic viscera free to act. Hence the old practice of administering an emetic early in a febrile disorder is, in

certain selected cases, a good one. Debility, whether general or especially cardiac, would forbid it. In a case of very feeble heart, known to the writer, death was occasioned by a paroxysm of vomiting. When antimony is used in the minute doses suggested, its effect should be carefully watched; in some cases it may be sufficient to reach the stage of nausea with its attendant flow from the respiratory mucous membranes.

According to Prof. Garrigou, the French hydrologist, many natural arsenical waters in France, Germany, and elsewhere contain also small quantities of antimony, although the analyses commonly quoted do not show its presence. He states that the warm springs of Bagnères de Bigorre and of Dax, in particular, have been resorted to with advantage in certain cardiac affections in which the quieting action of antimony upon the heart and respiration was desired.

Thus the history of a drug is always interesting because it reveals the trend of medical and therapeutic thought throughout the centuries. Slowly the empiricism is undermined and the application of a remedy often extremely valuable is discarded. The pendulum of therapeutic nihilism has obscured a drug that possesses undoubted value but such is the growth of medicine. A drug is now praised, now disclaimed, rising to a panacea, falling into obscurity and abandoned only to be *rediscovered* at some later date. On account of strong germicidal properties new interest in antimony arose. Today it finds application in the treatment of trypanosomiasis, kala-azar, schistosomiasis and many other exotic diseases.

In order to test the action of antimony on man, Mayerhofer subjected himself to experimental observation and to him a great deal of accurate description of its action is due.

Tartar emetic rose to an apogee in the time of Paracelsus in spite of the opposition by the orthodox medical authorities because it lacked the authority of Galen. Thus it appears that a valuable remedy was almost lost to medicine. Ackerman presented the following conclusions in regard to the action of antimony tartrate:

1. Quickens heart rate.
2. Weakens the heart, when its effects pass off heart rate increases.
3. Capillary and venous hyperemia.
4. Increased action of the skin (cold, clammy sweats and during this phase of action, warm sweats).
5. It first lowers temperature, then causes it to rise.
6. Augments intestinal gastric secretions (purging and vomiting).
7. Augments parotid secretion.

Antimony when applied to the skin in large amounts finds ready absorption followed by nausea, perspiration, profuse flow of the urine. The cause of the nausea is probably due to a gastrointestinal irritation due to its excretion through the bile and a stimulation of the central nervous system. Acute hepatic insufficiency may also result. The most exhaustive study of the chemotherapy of antimonial compounds has been carried out by Brahmachari of Calcutta. The author established that 55 milligrams of tartar emetic was the minimum lethal dose and *that subletlial doses did not give rise to any*

tolerance toward the drug except in very rare instances. The results also pointed to a cumulative action. These two considerations are of extreme importance from a therapeutic point of view particularly where destruction of protozoal organisms are involved.

Small amounts of tartar emetic may cause death and there are available in the literature many reports of deaths following its use. The action of antimony very closely resembles that of arsenic both therapeutically and as a chronic poison. A most important difference is the fact that in mammals tolerance does not develop; nor does arsenic habituation confer tolerance to antimony. This difference is explained by the observation that the absorption of antimony is not diminished; on the contrary, it is rather increased. Therapeutically antimony is used as a nauseant and an emetic. As an emetic its action is too slow and depressing. As a nauseant it has an advantage over all other metals since the dose is about one-tenth of that which produces vomiting. As an expectorant it is employed in the first stages of acute bronchitis.

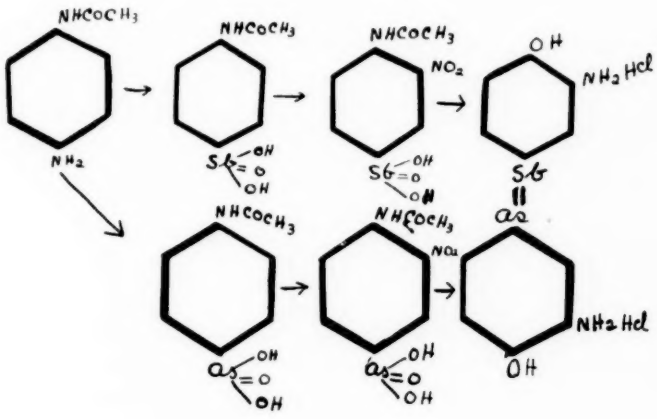
Organic trivalent compounds of antimony show action on protozoa similar to arsenic in the trivalent form. Organic antimonials have an advantage over organic arsenicals in that the parasites do not become immune and the trypanocidal activity is greater in the presence of serum, a condition which does not hold for the arsenicals.

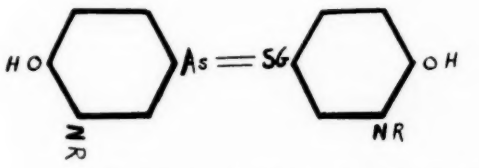
A vast amount of literature has appeared in which antimony has been successfully employed. Among these diseases are granuloma inguinale, chancroid, schistosomiasis, bilharziasis, trichinosis, filariasis, trypanosomiasis, leishmaniasis Kala Azar and Oriental sore, syphilis and leprosy and various filarial infections. Such is the clinical scope demanding new remedies to arrest and to cure the diseases. The toxicity and by-effects of tartar emetic were of such a nature that demand has been evident that new chemotherapeutic agents should be prepared.

Uhlenhuth, Mulzer and Hügel employed antimonials prepared under the supervision of Schmidt. A few of these preparations showed some promise but in many instances they showed an unsatisfactory relationship between toxicity and therapeutic index; that is to say, the toxicity was low and the therapeutic activity was low. This is a well-known physiologic principle that drugs of low toxicity invariably possess a low curative action in respect to protozoal infections. This is well illustrated in the case of the salvarsans and their substitutes. A sharply defined threshold is desirable, i.e., there must be a reaction between the drug and the parasites, and an absorption of the drug by the tissues of the host.

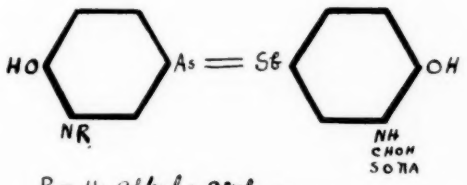
Further studies on antimonials were carried out by Brahmachari in India. These investigations have been most thorough and far reaching. It is safe to say that this author and his coworkers have given us our greatest knowledge on the action and excretion of antimony. From a point of view of scientific interest the articles are eminently worth reading. Rowntree and Abel presented a study of the antimony compound of thioglycolic acid in the treatment of experimental trypanosomiasis. Further studies on this group of compounds were published by one of us (M). The author also prepared antimony

lactate which was reported by Voegtlin and Smith from an experimental point of view. Among the next group of compounds studied were those in which antimony is attached to the benzene ring. These compounds are similar to arsenic in their chemical constitution and are roughly divided into pentavalent and trivalent preparations. This is also in the order of their therapeutic activity. The compounds are roughly represented by the following charts:





Arseno — Stibino compounds



R = H; alkyl; acyl sulfonyl, or other aliphatic group

Our studies have centered about the investigation of preparation M 303 in which arsenic and antimony in the trivalent condition exist in the same compound. It is readily evident that the compound presents a wide variety of possible substitution products, many of which have been studied in a cursory manner. The whole series have shown unusual prospects of therapeutic action. The dihydrochloride M 303 is a brownish yellow amorphous powder readily soluble in cold water and forms sodium salts which are suitable for intravenous administration. The experimental therapeutic test is shown in Tables I, II, and III as well as the toxicologic examination.

TABLE I

RAT NO.	SEX	INITIAL WT. 2/3	INJECTION WEIGHT	DOSE MG./KG.	AGE OF SOL. MIN.	DIED	FINAL WEIGHT	CONDITION
7569	M	114	125	130	15		121	
7570	M	112	124	*160*	20		122	
7571	M	114	128	110	23		137	
7572	M	109	123	130	28		120	
7573	M	98	112	*160*	31		100	
7574	M	114	129	110	35		134	
7575	M	98	113	130	38		111	
7576	M	114	125	160	41	6 hours		Hem. of lungs pleural exudate, some congestion of liver.
7577	M	104	113	110	46		114	

TABLE II

	SALVARSAN G-87			DISODIUM SALT				
				HOURS AFTER TREATMENT				
RAT NO.	WT. IN GRAMS	AS MG. PER KG.	TRYP. COUNT AT TIME OF TREATMENT	24 HR.	48 HR.	72 HR.	96 HR.	REMARKS
D.S.								
154	96	2	24,000	3,000	+++	Dead		Rats
155	97	3	80,000	6,600	+++	Dead		treated
156	80	3	78,000	7,000	+++	Dead		24 hr.
157	86	4.5	115,000	2,000	++	+++		after
158	70	4.5	151,000	4,000	++	+++		infection
159	83	7	109,000	–	–	+		*M. E. D.*
160	86	7	95,000	–	–	–		
161	88	9	53,000	–	–	–		
162	76	9	117,000	–	–	–		
163	87	1	98,000	88,000	++++	Dead		
164	83	1	118,000	54,000	++++	Dead		
165	78	1.5	82,000	7,000	+++	Dead		
166	74	1.5	127,000	4,500	+++	++++		
167	73	2.25	98,000	2,000	++	+++		
168	65	2.25	93,000	3,000	++	+++		
169	90	*3.5*	126,000	–	Trace	+		
170	69	*3.5*	123,000	–	–	–		*M. E. D.*
171	80	5	91,000	–	–	–		

1:46 Therapeutic coefficient.

Tabulated Collection of Several Protocols.

TABLE III

1. EXPERIMENTS WITH SODIUM SALT OF M-AMINO-P-URETHANOPHENYL-STIBINIC ACID

DATE:		9/16/1913		9/18/13	9/19/13	9/20/13	9/21/13
Sch.	Huhn	Nr. 841 inf.	0.1 St.	+	+ 0.1 St.	0	0
	Huhn	Nr. 842 inf.	0.1 St.	±	± 0.1 St.	0	0
H.	Huhn	Nr. 843 inf.		++ 0.1 St.	+ 0.1 St.	0	0
	Huhn	Nr. 844 inf.		++ 0.1 St.	+ 0.1 St.	0	0
C.	Huhn	Nr. 898 inf.		++	+++	Ex.	
	Huhn	Nr. 899 inf.		++	++++	Ex.	

2. EXPERIMENTS WITH M_1M^1-DIAMINO-P-OXYARSENOSTIBIOBENZOL

DATE:	10/4/1913		10/6/13	10/7/13	10/8/13	10/9/13
Sch. Huhn	Nr. 740 inf.	0.05 St.	0	0	0	0
Huhn	Nr. 741 inf.	0.05 St.	+	0	0	0
H. Huhn	Nr. 742 inf.		++ 0.05 St.	0	0	0
Huhn	Nr. 743 inf.		++ 0.05 St.	0	0	0
C. Huhn	Nr. 802 inf.		++	+++	Ex.	
Huhn	Nr. 803 inf.		++	+++	++++ Ex.	

3. EXPERIMENTS WITH M_1M^1-DIAMINO-P-OXY-P-CHLORARSENOSTIBIOBENZOL-DIHYDROCHLORIDE[1]

DATE:	10/22/1913		10/24/13	10/25/13	10/26/13	10/27/13
Sch. Huhn	Nr. 862 inf.	0.05 St.	0	0	0	0
Huhn	Nr. 863 inf.		+	0	0	0
H. Huhn	Nr. 864 inf.		++ 0.05 St.	0	0	0
Huhn	Nr. 865 inf.		++ 0.05 St.	+	0	0
C. Huhn	Nr. 866 inf.		++	++	+++	Ex.
Huhn	Nr. 701 inf.		+	++	+++	Ex.

Inf., plain infection (1 c.c. spirochete containing blood). St., the antimony preparation used. 0, no parasites found in a freshly prepared specimen. +, parasites found in a freshly prepared positive. Ex., Exitus letalis. Sch., control experiments. H., cures. C., controls without treatment.

From the foregoing discussion it has been pointed out that antimony has been a most valuable remedy in the treatment of disease. It was apparently forgotten for many decades and it seems probable that it will return to an active place in the therapeutic armamentarium. In a clinical paper a discussion of antimony in the treatment of leprosy will appear. Many investigations have shown the marked activity of antimony in the drying and healing of various ulcers. Several thousand injections of the new preparation M 303 have been given without the slightest inconvenience to clinician and patient, showing that it is well tolerated physiologically.

Preparation M 303 is a compound in which one molecule of nitro hydroxy phenyl arsinic acid is reduced with one molecule of nitro hydroxy phenyl stibinic acid. The resulting compound gives the combination of antimony and arsenic in the same preparation, each of the elements existing in the trivalent condition. This reduced compound is a product insoluble in ordinary solvents and is known as the base. This is treated with hydrochloric acid and a soluble preparation is obtained when the hydrochloride solution is precipitated from ether. The description of the product is found in U. S. Patent 1,422,294.

It is a preparation with a toxicity and a therapeutic activity with a most favorable ratio. Already its action in the treatment of syphilis and leprosy has shown most favorable indications of its value in diseases in which protozoal organisms are involved. It is a preparation more satisfactory than any now available on account of the favorable relation between the toxic dose and the curative action. On the basis of this relation, the fact that it contains arsenic and antimony in molecular proportions offers an entirely new thought in therapeutic application particularly in tropical diseases, leprosy and syphilis.

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THE PHOSPHORUS PARTITION IN NORMAL WHOLE BLOOD*

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UNTIL recently, the determination of blood phosphorus for diagnostic purposes has been confined to the inorganic phosphate fraction. There is, however, some evidence that the groups of organic phosphorus compounds occurring in the blood may not be entirely without clinical significance.

Kay[1] reported a decrease in the acid-soluble organic phosphorus of the blood, together with changes in lipin phosphorus, during acidosis following the ingestion of ammonium chloride. Byrom[2] observed in severe diabetes a marked decrease in the acid-soluble phosphorus, apparently the result of acidosis. The lipin phosphorus was increased in these same cases, in agreement with the findings of Bloor.[3] The decreased ester (acid-soluble organic) phosphorus could be restored to normal by insulin treatment. The increased lipin phosphorus was found to be more resistant, possibly on account of the abnormally large amount of fat in the diabetic diet.

Gröbly[4] based a diagnosis of malignant disease on the total phosphorus content of the blood, related to the erythrocyte count. This "phosphorus quotient" was supported by Vorschütz and Vorschütz[5] and by Zerner,[6] but was found entirely valueless by Buckman, Minot, Daland and Weld[7] and by Byrom and Kay.[8] The latter authors, in the course of their study, demonstrated that the high "phosphorus quotients" frequently observed in cancer patients could be attributed to the concurrent anemia, and that equally high quotients could be obtained with patients free from malignant disease, but suffering from some condition which would cause an increase in the organic or lipin phosphorus (e.g., nephritis or jaundice). Marked changes in the inorganic phosphorus fractions were noted in anemia, polycythemia and leucemia, largely associated with the changes in the proportions of the blood cells. Wohlfarth[9] had also decided that the change in the phosphorus content of the corpuscles was associated with cachexia, not necessarily of malignant disease.

Buckman, Daland and Weld[10] have studied the total phosphorus of the blood in cases of myelogenous leucemia, finding a correlation with the number of immature myeloid cells and to a lesser degree with the basal metabolic rate.

In renal disease, in addition to the already recognized changes in inorganic and lipin phosphorus, Byrom and Kay[11] recognized characteristic changes in the acid-soluble organic fraction, leading them to the conclusion that there are definite changes in both the amount and nature of the phosphorus of the erythrocytes.

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Disturbances in the distribution of the organic phosphorus of the blood in tuberculosis have been studied by McCluskey[12] and by Morel and Aalam.[13]

Comparison and correlation of the figures for normal and abnormal phosphorus partitions from the above sources are made difficult by the variety of methods used by the different workers. Many of the methods used have been rendered obsolete by recent improvements. The purpose of this present work is to establish the normal range of values for the groups of phosphorus compounds found in whole blood, using a modern colorimetric method simple and accurate enough for clinical application, these normal values to serve as a starting point for the study of the phosphorus partition in certain abnormal conditions.

Kay and Byrom[14] have published a similar study of 31 normal cases, using a modification of Briggs'[15] method for phosphorus determinations. The method of Fiske and Subbarow[16] offers certain simplifications in technic and refinements in accuracy as compared with that of Briggs and appears to be the method of choice, in this country at least, of the majority of workers. In this study we have used the method of Fiske and Subbarow throughout, making only such alterations in the original procedure as were necessary to make it applicable to the material in hand.

The reagents necessary for the Fiske and Subbarow method for phosphorus determinations in blood (or urine) are as follows:

10 N Sulphuric Acid. 450 c.c. of concentrated sulphuric acid added to 1300 c.c. of water.

Molybdate I. 2.5 per cent ammonium molybdate in 5 N sulphuric acid.

Molybdate II. 2.5 per cent ammonium molybdate in 3 N sulphuric acid.

Molybdate III. 2.5 per cent ammonium molybdate in water. "As soon as any considerable amount of sediment (ammonium trimolybdate) has appeared in this solution, it should be discarded."

10 per cent trichloracetic acid. The trichloracetic acid used should be free from phosphate or from color-inhibiting impurities. In our work, we use trichloracetic acid which has been redistilled in vacuo.

Standard Phosphate Solution (5 c.c. = 0.4 mg. P). A solution of pure monopotassium phosphate (0.3509 grams per liter) made up in approximately tenth normal sulphuric acid.

Aminonaphtholsulphonic acid reagent. Dissolve 0.5 grams of purified 1, 2, 4-Aminonaphtholsulphonic acid in 500 c.c. of 5 per cent sodium bisulphite to which has been added 5 c.c. of 20 per cent sodium sulphite. This solution is stable for about two weeks if well stoppered. For method of purification of the technical acid, one should consult the original paper of Fiske and Subbarow.[16]

The technic of the various determinations will be described by us in discussing the individual fractions. For a more detailed treatment, the original paper should again be consulted.

The subjects who kindly contributed the blood for this series of analyses were first-year medical students, 47 men and 6 women. All were in good health, as shown not only by their continued presence in medical school, but also by a thorough physical examination and series of vital function tests prescribed as part of the work of the first year. All blood specimens were taken in the morning before breakfast. Lithium oxalate was used as the anticoagulant.

INORGANIC PHOSPHATE

This determination was carried out on a trichloracetic acid filtrate of whole blood, prepared immediately after the blood was taken. This prompt preparation of the filtrate is necessary for accurate determinations of inorganic phosphate in whole blood, as pointed out by Martland and Robison.[17] The trichloracetic acid used in preparing the filtrates was redistilled in vacuo to remove reducing substances (Martland and Robison, Fiske and Subbarow). The procedure was as follows (following Fiske and Subbarow):

Twenty c.c. of 10 per cent trichloracetic acid was pipetted into a 100 c.c. Florence flask. To this was added from an Ostwald pipette 5 c.c. freshly drawn whole blood, the addition being made slowly and with gentle shaking. The flask was then stoppered and the contents mixed thoroughly, then filtered through an ashless paper (Whatman No. 40). This filtrate served for determinations of inorganic and acid soluble organic phosphorus.

For the determination of inorganic phosphate, 5 c.c. of the filtrate was measured into a 10 c.c. volumetric flask. One c.c. of Molybdate II was then added, followed by 0.4 c.c. of the aminonaphtholsulphonic acid reagent. The contents of the flask were made up to 10 c.c. volume with water and mixed. After five minutes standing for color development, the blue solution was read in a Duboseq colorimeter against a standard (set at 20) prepared as follows: To a 100 c.c. volumetric flask containing about 65 c.c. water add 5 c.c. of the standard phosphate solution (5 c.c. = 0.4 mg. P), 10 c.c. Molybdate I and 4 c.c. of the aminonaphtholsulphonic acid reagent. Make up to volume and mix. This standard should be prepared simultaneously with the unknown. With the standard set at 20, eighty divided by the reading will give the milligrams of inorganic phosphate phosphorus per 100 c.c. blood.

Fifty-two determinations of inorganic phosphate were made in all. The maximum value was 4.7 mg. (as P) per 100 c.c. whole blood, the minimum 3.0 and the mean 3.8 mg.

ACID-SOLUBLE PHOSPHORUS

This determination was carried out on the same trichloracetic acid filtrate, using 5 c.c. and running duplicate estimations. To a Pyrex test tube graduated at 50 c.c. and not previously used for nonprotein nitrogen determinations, 5 c.c. of the filtrate and 2.5 c.c. of 10 N sulphuric acid were added. The tubes were then heated in a beaker of boiling water until the water was nearly driven off, as shown by incipient charring of the contents. The tubes were then removed from the water-bath, the outside dried with a cloth and the digestion completed with a micro-burner. A low flame was used, and kept 2 cm. below the tube. This gentle heat was applied until charring appeared to be complete, then one drop of concentrated nitric acid was added down the wall of the tube, which in most cases turned the solution quite colorless. If not, another drop was used. The gentle heating was continued for half a minute after the addition of nitric acid, and the tube then allowed to cool. Thirty-five c.c. of water, 5 c.c. of Molybdate III, and 2 c.c. of the aminonaphtholsulphonic acid reagent were added, the contents made up to 50 c.c. volume, allowed to stand five minutes and then read against the same standard as described for inorganic phosphate. Four hundred divided by the reading (standard at 20) gives the milligrams of acid-soluble phosphorus per 100 c.c. blood.

Fifty-two duplicate analyses were made. The maximum value was 31 mg. per 100 c.c. blood, the minimum 22.1 and the mean 26.7 mg.

ACID-SOLUBLE ORGANIC PHOSPHORUS (ESTER PHOSPHORUS)

This figure was obtained in each case by subtracting the inorganic phosphate phosphorus from the acid soluble phosphorus. The maximum value was 29.4 mg. per 100 c.c. blood, the minimum 18.7 and the mean 22.9 mg. We did not carry through in this series the partition of this fraction into hydrolysable and nonhydrolysable ester phosphorus, as done by Kay and Byrom.[14]

LIPIN PHOSPHORUS ("LECITHIN" PHOSPHORUS)

The alcohol-ether extracts were made according to Bloor, Pelkan and Allen,[18] using 5 c.c. whole blood and making up to 100 c.c. volume with the alcohol-ether mixture. The preparation of the alcohol-ether extract of blood is as follows: to 60 c.c. of an alcohol-ether mixture (75 c.c. redistilled alcohol and 25 c.c. ether) in a 100 c.c. volumetric flask are added 5 c.c. blood slowly and with shaking. The flask is then heated just to boiling in a beaker of boiling water. When cool it is made to volume with alcohol-ether mixture, mixed and filtered. For each analysis 10 c.c. of the alcohol-ether extract (equivalent to 0.5 c.c. blood) was evaporated to dryness in a Pyrex test tube graduated at 25 c.c., using a boiling water-bath. The dry residue was treated with 5 c.c. of 5 N sulphuric acid and digested over a micro-burner. When charring had taken place, two or three drops of concentrated nitric acid sufficed to produce a clear solution. After cooling and dilution, 5 c.c. of Molybdate III (Fiske and Subbarow) and 2 c.c. of the reducing agent were added, and the volume made up in the tube to 25 c.c. This solution was read in the colorimeter against the usual standard. This procedure for "lecithin" phosphorus is the one used by McClure and Huntsinger.[19]

Thirty-two determinations (in duplicate) were made of the lipin phosphorus. The maximum value was 16.8 mg. per 100 c.c. blood, the minimum 11.8 and the mean 14.2 mg.

TOTAL PHOSPHORUS

For this determination, one c.c. of blood was digested with 5 c.c. of 10 N sulphuric acid in a 200 × 25 mm. Pyrex test tube. Nitric acid was added, a few drops at a time, until the contents of the tube were clear and nearly colorless (a faint yellow tinge remained after long digestion, but disappeared on cooling). The first part of the digestion was done on a sand-bath, several tubes at a time. The digestion was completed by heating the tubes individually over a micro-burner. After cooling, the digest was washed into a 100 c.c. volumetric flask, 10 c.c. of Molybdate III (Fiske and Subbarow) and 4 c.c. of the reducing agent added, the contents of the flasks made up to volume and read against the usual standard.

Forty-seven determinations of total phosphorus were made (in duplicate) the values ranging from a maximum of 44.4 mg. per 100 c.c. blood to a minimum of 34.9 mg., with a mean of 39.2 mg.

THE PHOSPHORUS PARTITION

If we calculate the percentages of the total phosphorus made up by the various groups of compounds, we find that the inorganic phosphate accounts for from 7.8 to 12.3 per cent of the total, with a mean value of 9.7 per cent.

The acid soluble phosphorus forms from 61.8 to 76.0 per cent of the total, with a mean at 68.1 per cent.

The acid soluble organic phosphorus, or ester phosphorus, composes from 53.3 to 66.5 per cent, with 58.3 per cent as a mean value.

The lipin or "lecithin" phosphorus makes up from 30.8 to 42.2 per cent of the total phosphorus, with its mean percentage at 36.0

There is no residual, or unaccounted phosphorus according to this grouping, although the situation is not strictly analogous to the nitrogen partition. In the phosphorus partition, we do not in any case determine individual compounds, but merely group the phosphorus containing substances according to solubility.

If the partition into acid-soluble compounds and those soluble in alcohol-ether is sharp, then the sum of these fractions should equal the total phosphorus. Out of the 31 cases in which we made complete analyses, there was agreement to within one milligram per 100 c.c. blood in 15 cases, or nearly one-half. In the remaining 16 cases, the sum of the acid-soluble and lipin fractions was greater than the actually determined total phosphorus by an amount varying from 1.4 to 4.6 mg. per 100 c.c. The mean of the differences of the entire group of 31 cases was 1.7 mg. In no case was the sum of the acid-soluble and lipin phosphorus less (by more than 0.3 mg.) than the total phosphorus by direct analysis.

This failure of the found and calculated total phosphorus to coincide exactly may indicate that a small amount of phosphorus may be soluble both in trichloracetic acid and in the alcohol-ether mixture. It should be considered, however, that in the determination of this "extra" or "ambisoluble" phosphorus, the analytical errors of three determinations are added and that this deviation may simply represent their algebraic sum.

Table I gives a summary of the findings detailed above.

The values we obtain for total phosphorus are in agrement with those obtained by Bloor[20] and by McCluskey[21] who used a modification of Bloor's

TABLE I

PHOSPHORUS PARTITIONS IN NORMAL WHOLE BLOOD*

	NUMBER OF CASES	MAXIMUM VALUE	MINIMUM VALUE	MEAN VALUE
Inorganic phosphate phosphorus	52	4.7	3.0	3.8
Per cent of total phosphorus		12.3%	7.8%	9.7%
Acid soluble phosphorus	52	31.0	22.1	26.7
Per cent of total phosphorus		76.0%	61.8%	68.1%
Acid soluble organic phosphorus	51	29.4	18.7	22.9
Per cent of total phosphorus		66.5%	53.3%	58.3%
Lipin phosphorus	35	16.8	11.8	14.2
Per cent of total phosphorus		42.2%	30.8%	36.0%
Total phosphorus by analysis	47	44.4	34.9	39.2
Total phosphorus by addition	31	47.2	35.2	39.8

*Values are given in milligrams phosphorus per 100 c.c. whole blood. Percentages are calculated on the total phosphorus by analysis.

method. Smith and Brown[22] report values for total phosphorus between 55 and 60 mg. per 100 c.c. whole blood. The values obtained by Kay and Byrom[14] run very slightly lower than ours.

Our values for acid-soluble and ester phosphorus are in close agreement with those of Kay and Byrom[14] and higher than those of McCluskey.[21] Our values for lipin or "lecithin" phosphorus agree with those obtained by McClure and Huntsinger[19] but tend to run slightly higher than those of Kay and Byrom[14] or McCluskey.[21]

SUMMARY

Analyses were made of whole blood from healthy fasting young adults for total phosphorus, acid-soluble, inorganic, acid-soluble organic and lipin phosphorus. The results are summarized in Table I.

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PHOSPHORUS METABOLISM*

II. The Distribution of Phosphorus in Normal and Cancer Bloods

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A. NORMALS

While much advance has been made in the study of human blood phosphorus during the last fifteen years, both qualitatively and quantitatively, comparatively little work on the quantitative distribution in single samples of blood in health and disease has been made except by Byrom and coworkers. Byrom's fourth and latest paper[1] is on the phosphorus content of the blood in diabetes mellitus. The determinations made are those of inorganic, ester, and lipid phosphorus (and hematocrit) on whole blood. The paper of Stanford and Wheatley,[2] while reporting determinations on whole blood, corpuscles and plasma, contains figures on only five normal cases and the figures are doubtless too variable to represent the true values for normal bloods.

To refresh the reader we enumerate below the phosphorus compounds now generally accepted to be present in blood:

Inorganic phosphates.
Carbohydrate esters of phosphoric acid.
Phospholipids: Cephalin, sphingomyelin and lecithin.
Nucleic acid.
Adenine nucleotide (and possibly pyrimidine nucleotide).
Phosphoglyceric acid.

While it is not possible or feasible by the use of present methods to determine the amounts of these compounds separately, we can, as is usually done, divide them into groups and determine these in corpuscles and plasma or serum and find the quantitative distribution in that way in health and disease. In this research we have done that, using the analytical methods described in the preceding paper.[3]

In order to determine the degree of anemia and to check up on the accuracy of our work, we determined the cell volumes of the bloods by using graduated centrifuge tubes when separating corpuscles and plasma, allowing the usual half hour of centrifuging at about 2200 r.p.m. We were aware that this method is not so accurate as the hematocrit.

With all of the normal bloods, centrifuging was begun immediately after drawing. In the cancer bloods as much as three-fourths of an hour elapsed be-

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fore centrifuging could be begun, on account of the time taken to bring the samples from the City Hospital to our laboratory. The anticoagulant used was potassium oxalate (0.2 per cent). While we have not made any experiments in that direction, there is no doubt that there is some change in the distribution of some of the phosphorus compounds as the blood is kept after drawing and is handled in analysis. We have reviewed the papers of Martland, Hansman and Robison,[4] Lawaczweck,[5] and Kay and Byrom[6] on this problem. The changes are difficult to obviate entirely; but we believe that there is not enough of a change to affect our results appreciably.

To establish normal limits and averages we present Table I, being the results of analyses of 21 bloods from students and members of the staff of this department. The samples were drawn between two and three hours after customary breakfasts and were treated as described under the System of Analysis.

It has been shown by Kay and Byrom that there is only a slight variation in values due to alimentary conditions. The variations are so small that we believe that they have no bearing on our results and we have therefore disregarded them.

Subjects Nos. 1 and 4 were persons of about forty-five years of age. Nos. 9 and 20 were between thirty and thirty-five years old; all of the remaining being between twenty and thirty years of age. All were males except No. 1. The table arrangement is in order of decreasing total phosphorus in whole blood. While the average values found for different determinations agree well with most values found by other workers, the following comments are given:

1. The corpuscles contain on the average 4.4 times as much total phosphorus as plasma.

2. The corpuscles contain only 7.3 per cent less of inorganic phosphate phosphorus than does the plasma.

3. The ester phosphorus occurs in relatively large amount in the corpuscles. It is not more variable than some of the other phosphorus fractions.

4. The lipid phosphorus is 17 per cent greater in the corpuscles.

5. We found on the average 18 per cent of the phosphorus of the plasma that is not in lipid or phosphate form. It is commonly believed to be carbohydrate esters of phosphoric acid, and there is some evidence for this. While some have come to the conclusion that there is no such fraction in the plasma or serum, others report various amounts, as is shown in tabular form by Martland and Robison.[7] All values for this fraction as found by others and by us, result from a calculation by difference and since the quantity is very small and appears to be so variable, exact values are perhaps yet to be elucidated. Although slight hemolysis may play an important rôle here in permitting some of the corpuscular ester phosphorus to pass into the plasma, in our work we tried to avoid this so far as possible in that the bloods were analyzed as quickly as possible and any blood which showed visible sign or suspicion of hemolysis was discarded. We did not avoid some loss of carbon dioxide, but we exposed the samples to the air as little as possible.

TABLE I

ON THE DISTRIBUTION OF PHOSPHORUS IN NORMAL HUMAN BLOODS

SUBJECT NO.	CORPUSCLE VOLUME	TOTAL P. PER 100 C.C. OF WHOLE BLOOD	PHOSPHORUS PER 100 C.C. OF CORPUSCLES				PHOSPHORUS PER 100 C.C. OF PLASMA			
			TOTAL	LIPID	INORGANIC PHOSPHATE	TOTAL ACID-SOLUBLE	TOTAL	LIPID	INORGANIC PHOSPHATE	TOTAL ACID-SOLUBLE
	per cent	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	42.4	39.2	74.5	7.57	3.40	66.9	16.00	11.76	2.56	3.92
2	50.7	38.0	61.2	10.90	2.81	49.3	14.20	8.70	4.03	4.85
3	53.3	37.9	62.0	12.40	3.70	49.0	14.80	9.60	4.28	5.10
4	43.3	37.6	70.3	13.50	4.40	56.7	15.00	11.60	4.43	4.62
5	50.4	37.4	59.3	10.75	4.00	46.5	13.26	9.26	3.92	4.13
6	51.9	37.2	59.2	12.19	1.08	46.3	11.82	8.37	3.54	3.97
7	42.3	36.5	64.5	12.40	3.84	49.7	12.00	7.19	4.15	4.56
8	46.6	36.4	59.6	12.20	4.69	47.4	15.90	9.95	4.35	4.93
9	47.7	36.4	61.3	10.30	3.44	50.0	13.04	9.43	3.05	4.59
10	48.6	36.0	57.1	10.47	4.02	45.0	13.57	10.42	3.01	3.55
11	49.1	35.9	59.8	10.20	3.15	49.0	14.54	10.00	4.30	4.22
12	42.0	35.1	61.5	10.69	3.01	47.8	12.00	7.33	3.10	4.78
13	47.0	34.6	59.4	7.75	3.75	52.9	12.88	9.17	4.08	3.99
14	51.4	34.5	54.4	10.80	2.90	47.4	12.94	9.80	3.60	3.64
15	50.7	34.3	52.4	9.76	2.38	42.7	10.86	8.33	3.12	3.49
16	49.6	32.5	53.1	10.15	2.10	45.0	12.10	6.94	4.20	6.62
17	50.9	32.2	53.5	10.47	4.00	42.0	11.80	8.62	3.65	3.90
18	50.0	32.1	46.3	9.95	1.92	36.1	10.86	7.14	3.90	4.20
19	46.7	30.8	54.6	10.58	2.84	43.5	11.68	7.46	3.47	3.86
20	46.0	29.8	53.8	10.47	3.77	42.9	12.82	10.05	3.36	3.36
21	36.0	28.6	60.6	9.57	4.52	49.3	14.28	8.85	4.34	5.24
Highest	53.3	39.2	74.5	13.50	4.69	66.9	16.00	11.76	4.43	6.62
Lowest	36.0	28.6	46.3	7.57	1.08	36.1	10.86	6.94	2.56	3.36
Average	47.5	34.9	58.1	10.63	3.32	48.0	13.01	9.05	3.73	4.36

B. CANCER CASES

In their third paper on blood phosphorus in health and disease Byrom and Kay[8] have stated briefly the current findings of blood phosphorus in cancer. The reader is referred to it. They as well as Buckman, Minot, Daland and Weld[9] conclude that there is no significant change of the phosphorus distribution in this disease, although in previous work by others, conclusions were reached tending to show a definite change in the distribution, notably an increase in total phosphorus in whole blood, which was more pronounced in the corpuscles. Although Byrom and Kay's work seemed convincing, we still wanted to consider values obtained when both corpuscles and plasma were determined separately. We present therefore Table II, being results of analyses of 15 bloods from advanced cancer cases from the Buffalo City Hospital.

The bloods were drawn just before breakfast, oxalated, and taken to our laboratory without delay, where they were analyzed in the same way as the normals. The cases in the table are arranged in order of decreasing total phosphorus in the whole blood. All of the subjects were men except Nos. 1, 4, 5, and 13, who were women.

It has been shown by Kay and Byrom[6] that there are only slight differences in blood phosphorus distribution due to sex and no differences due to age. We do not hesitate therefore to compare cancer bloods from persons who are on the average considerably older than our normals.

It is to be noted first that some of the patients were anemic, the corpuscle volume ranging from 22.7 to 48.1 per cent. There is no correlation of the corpuscle volume with age such as seemed possible in the normal cases. Neither do we find any relation between corpuscle volumes and total phosphorus in the whole blood.

Comparing the cancer bloods with the normals it is seen that the total phosphorus in the whole blood is 8.9 per cent less in the cancer cases. While this difference is not great, the explanation is most likely the effect of anemia. Byrom and Kay find that the cancer cases with anemia gave an average of 35.5 mg. per cent total phosphorus as against 39.8 mg. for their nonanemic cancer cases. Our findings are opposed to those of Gröbly[10] and others who found a considerable increase in total phosphorus even in only suspected cancer cases, and are more in agreement with Byrom and Kay.

Comparing the corpuscle total phosphorus, our average for the cancer cases is 68.4 mg., average for normals 58.1; thus there is 17.8 per cent more in the former. This seems significant. It was found and emphasized by Gröbly. Byrom and Kay do not determine this definitely because they did not analyze corpuscles and plasma separately, but they "not infrequently" found high phosphorus quotients in cancer cases, which they attribute to concurrent anemia. We cannot interpret our results as being due to anemia. When we examine the corpuscle fractions we see that it is distinctly the total acid-soluble (which is mostly ester) and lipid phosphorus that are increased in nearly the same proportion. Byrom and Kay believe that the ester phosphorus is an integral part of the corpuscular furniture because the esters are extruded from the corpuscle only on hemolysis. We do not believe that

TABLE II

ON THE DISTRIBUTION OF PHOSPHORUS IN CANCER BLOODS

NO. SUBJECT	CORPUSCLE VOLUME	TOTAL P PER 100 C.C. OF WHOLE BLOOD	PHOSPHORUS PER 100 C.C. OF CORPUSCLES				PHOSPHORUS PER 100 C.C. OF PLASMA			
			TOTAL	LIPID	INORGANIC PHOSPHATE	TOTAL ACID-SOLUBLE	TOTAL	LIPID	INORGANIC PHOSPHATE	TOTAL ACID-SOLUBLE
	per cent	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	35.4	39.8	87.9	13.16	2.10	73.1	13.40	8.80	4.54	4.71
2	48.1	36.7	66.7	11.90	1.72	51.5	12.64	8.84	3.35	3.80
3	46.8	34.4	60.6	10.87	3.12	51.8	11.69	6.84	3.74	3.97
4	45.4	34.4	60.6	12.20	2.80	47.0	14.65	8.51	4.76	5.25
5	29.7	33.6	72.0	11.90	2.33	59.0	13.60	7.50	4.27	4.65
6	22.7	33.0	80.8	12.20	2.50	66.5	13.00	7.66	4.15	4.65
7	29.5	32.6	83.0	16.80	2.80	64.5	11.62	7.57	4.34	4.50
8	40.2	32.0	74.0	11.76	2.78	64.1	10.33	7.18	3.57	3.51
9	39.6	29.8	57.7	10.15	3.07	46.8	11.44	7.38	3.13	3.66
10	43.2	29.6	59.0	11.43	2.95	48.3	10.70	6.25	3.52	4.33
11	36.9	28.8	62.7	12.05	1.25	48.3	11.44	7.69	3.12	3.20
12	36.4	28.6	57.4	14.08	2.47	46.7	10.29	8.79	3.92	4.38
13	25.6	28.2	76.9	10.75	1.47	70.9	11.83	6.78	3.20	3.35
14	32.7	28.0	63.0	14.00	2.42	49.0	12.12	9.04	3.51	3.70
15	34.0	27.6	64.2	13.20	2.48	55.0	9.04	5.79	2.81	2.98
Highest	48.1	39.8	87.9	16.80	3.12	73.1	14.65	9.04	4.76	5.25
Lowest	22.7	27.6	57.4	10.15	1.25	46.7	9.04	5.79	2.81	2.98
Average	36.4	31.8	68.4	12.43	2.42	56.1	11.85	7.64	3.72	4.05

their conclusion is correct. From their standpoint one might argue that potassium chloride, characteristic of the corpuscles, is also structural, but this we know is not the case. Also the composition of the esters as carbohydrate esters of phosphoric acid would lead one to believe that they are metabolic substances. But we have no plausible explanation for the greater concentration of ester. One possibility, however, may be that if the carbohydrate esters of phosphoric acid represent intermediary compounds in metabolism, the removal phase from the corpuscles may exhibit a lag under certain conditions.

Comparing the lipid phosphorus in corpuscles and plasma of cancer bloods we find distinctly more in the corpuscles, corpuscles 12.4, plasma 7.6 mg. In the normal bloods the average is: corpuscles 10.6, plasma 9.05 mg. The cancer bloods have, then, 17.2 per cent more, the plasma 15.6 per cent less lipid phosphorus than the normals. Is this difference metabolic or structural? It cannot be accounted for by a difference in size of the corpuscles in these two series of bloods; that difference, if any, must be small. It cannot be due to anemia because if we choose some of the nonanemic cancer bloods, e.g., Nos. 3, 4, 10, the average is: corpuscle lipid phosphorus 11.5 mg., plasma lipid phosphorus 7.2 mg. While the corpuscle lipid phosphorus of 11.5 mg. stands midway in value between the average for all cancer cases and the normals, the plasma lipid phosphorus value of 7.2 mg. does not approach the average normal values but is distinctly lower.

It is very doubtful that it can be due to emaciation if we consider that in this condition we have a low level of nutrient material, in this case phospholipid, in the blood, because persons having a corpuscle volume of 45 per cent are not emaciated. But the differences are perhaps not so significant when we see that a number of normal bloods show just as much or little lipid phosphorus. We are not inclined to emphasize a characteristic difference.

In reference to the inorganic phosphate and the total acid-soluble phosphorus we find no significant difference in cancer bloods versus normal bloods, except in the case of the inorganic phosphate in the corpuscles. Here we find 30 per cent less in the cancer cases. We know of no reason why inorganic phosphate phosphorus should be less in cancer corpuscles. It can hardly be due to a migration between corpuscles and plasma because both bloods were treated alike; and our hospital patients in general do not show an inorganic phosphate that is lower than normal.

We are indebted to the students, staff members, and the Buffalo City Hospital for cooperating in furnishing blood samples.

SUMMARY AND CONCLUSIONS

The distribution of phosphorus in 21 normal and in 15 cancer bloods is given; the analyses include determinations on both corpuscles and plasma.

Cancer bloods exhibit some distinct differences from normal bloods in phosphorus distribution. In the corpuscles: the ester phosphorus is increased 20.5 per cent, the lipid phosphorus 17.2 per cent, while the inorganic phos-

phate phosphorus is decreased 30 per cent. In the plasma: the lipid phosphorus is decreased 15.6 per cent.

It is yet doubtful whether any of these changes are characteristic for cancer blood; other pathologic bloods must first be studied.

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AN INSTANCE OF THE TRANSFUSION OF INCOMPATIBLE BLOOD WITHOUT REACTION, AND A SOURCE OF ERROR DUE TO CONTAMINATION OF GROUPING SERA WITH A MUSTARD BACILLUS*

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THE transfusion of isoagglutinable blood into a recipient whose blood contains the corresponding isoagglutinins is known to be usually followed by serious symptoms, which sometimes are fatal. In a few reported instances, however, in spite of the incompatibility of the transfused blood, the transfusion of the usual quantity of blood was performed without causing even alarming symptoms.

In a serologic study of one such case, Burnham[1] found that the absence of symptoms was due to the fact that the isoagglutinins in the Group B recipient's blood were incapable of clumping the Group A donor's blood cells at body temperature.

In Burnham's case, the incompatible A corpuscles were demonstrable in the recipient's blood on the third day following the transfusion, but not on the sixth day. He states that there was no hemoglobinuria at any time. The mistake in the grouping in his case was due to the use of a very weak suspension of cells together with a failure to stir the test mixture sufficiently.

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It is the purpose of this paper to describe another instance of the transfusion of incompatible blood which was not followed by any symptoms; and also to report a hitherto undescribed source of error in the blood grouping test.

The patient, a child of eight years, by the original test seemed to belong to Group B, and a Group B donor was supplied from the Donor Bureau of the Blood Transfusion Betterment Association. Through an accident, the direct matching of the two bloods was omitted.

The patient received 300 c.c. of the Group B blood without exhibiting the slightest symptom at any time. There was no hemoglobinuria.

Later on, the same day, it happened that the supposed Group B blood of the patient was tested and several Group A sera, all of which failed to cause clumping, indicating that the blood was not of Group B, but of Group O.

We then obtained the anti-B serum with which the patient's blood had been originally tested and discovered that that specimen of serum clumped all human blood including Group A. Since this serum was the same as that used in the Bureau, which was known to be group specific, it was assumed that its nonspecific action was due to bacterial contamination. Cultures were therefore made and a pure growth of a chromogenic gram negative bacillus was obtained, which was identified by George W. Wheeler as "a mustard bacillus." This organism was found to be capable of inducing the nonspecific agglutinating property described above, when it was inoculated into anti-A serum or into guinea pig serum which had been deprived, by absorption with washed human blood cells (Group O), of its normal heteroagglutinin. In nutrient broth or crystalline egg albumen inoculated with the mustard bacillus, no agglutinating property for human corpuscles developed. The strain produced no acid in any of these media.

The following ten strains of bacteria were found incapable of inducing an agglutinating property for human corpuscles in absorbed guinea pig serum:

1. B. alkaligenes
2. B. coli
3. B. hoffman
4. B. lactus aerogenes
5. B. prodigiosus
6. B. proteus
7. B. subtlis
8. B. xerosis
9. Staphylococcus albus
10. Staphylococcus aureus

The blood grouping test can be interfered with in another way by bacterial contamination. O. Thomsen[2] has reported that human blood corpuscles may be changed by a certain infective agent, in such a way that they are nonspecifically agglutinated by any normal human serum.

In view of these and other sources of error in the very serious test of blood grouping, it seems necessary to lay down the following precautionary rules:

1. The blood specimen (unless sterile) should be grouped on the day it is obtained.
2. The grouping with known test sera should be checked by testing the patient's serum against known A. and B. corpuscles.

The only reasonable provisional explanation of the complete absence of symptoms upon the transfusion of the incompatible blood, was either that the β isoagglutinin was absent in the recipient's blood (a very rare occurrence) or (as in Burnham's case) it was present but incapable of acting at body temperature. The latter explanation proved to be the correct one.

A specimen of blood was obtained from the patient on the day following the transfusion; it was defibrinated by stirring and centrifugalized while still warm. The serum clumped both A and B corpuscles on the slide, at room temperature (A cells somewhat more strongly than B). In the test tube, at 42°, 0.2 c.c. of the serum was incapable of producing any agglutination of 0.2 c.c. of a 2 per cent suspension of either A or B corpuscles, observed over one hour. The same mixtures at 0° C. showed immediate strong agglutination of cells in both instances. Examination of the corpuscular sediment of this specimen of the patient's blood showed the presence of the B cells when the blood was tested with anti-B serum; the appearance of the clumps was the same as that described by Burnham, a few large clumps among many entirely unaffected cells. On account of the condition of the patient, a further specimen could not be obtained until the seventh day after the transfusion, at which time the B corpuscles had disappeared. At this time, she was recovering from her illness.

SUMMARY

1. An eight-year-old Group O patient was given a transfusion of 300 c.c. of Group B blood without at any time exhibiting the slightest reaction.

2. The absence of symptoms was apparently due to the inability of the β isoagglutinin to clump B corpuscles at body temperature.

3. The transfused B corpuscles had completely disappeared by the seventh day.

4. The transfusion of the incompatible blood was made on account of a mistake in the grouping of the patient due to contamination of the anti-B grouping serum with a "mustard bacillus," which produces a nonspecific agglutinating property for all human blood cells.

5. A routine check of the usual method of grouping with known test sera is recommended, by testing the patient's serum against freshly obtained A and B corpuscles, and also against Group O corpuscles.

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THE CONGO-RED TEST WITH SPECIAL REFERENCE TO EXCRETION OF THE DYE IN THE URINE*

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CONGO-RED is an organic dye whose full chemical name is sodium-benzidene-diazo-binaphthylamine-sulphonate. Its molecular weight is 696, a fairly large molecule. It is soluble in water to the extent of a little more than 1.5 per cent, forming a clear deep red solution considered by most chemists to be colloidal. When the hydrogen-ion concentration of a solution of Congo-red is raised to between P_H 3 and 5, an acid is formed from the sodium salt and the solution becomes a deep blue, the dye then slowly precipitating out in solid form. In alkaline or neutral solution the greater part of the dye passes through ordinary filters but it will not pass through a collodion membrane by either ultra-filtration or dialysis. Recent chemical studies indicate that when Congo-red in solution is added to an albumin solution either in vivo or in vitro it becomes adsorbed to the albumin. When adsorbed to serum albumin in vivo it is slowly removed by the liver and excreted in the bile.

Congo-red has been used in medicine for determinations of blood volume by Griesbach, and by Rowntree and Brown, who have shown that in clear sterile fresh solutions in triple-distilled water it can be injected into the blood stream in amounts as large as 300 mg. without any untoward effect on the patient. In three to six minutes after injection it becomes completely and uniformly diffused throughout the blood stream and is then gradually eliminated over a period of from twenty-four to forty-eight hours.

Bennhold, in 1923, described his Congo-red test as a diagnostic procedure. His method was to inject 10 c.c. of a 1 per cent solution, or 15 c.c. of a 0.75 per cent solution intravenously and withdraw samples of blood four and sixty minutes afterward, determining the comparative content of dye in the serum with a colorimeter. He found that in normal subjects from 11 to 29 per cent (average 19 per cent) of the dye disappeared in this one hour period. By duodenal drainage and studies in a case of biliary fistula he concluded that almost all of the dye was normally excreted in the bile. He could not recover it from the urine in normal subjects except in minute traces. In cases of glomerulonephritis and nephrosclerosis he found that the dye disappeared from the blood stream at approximately the normal rate, whereas in some cases of disease of the liver the rate of disappearance was definitely slowed, although not in proportion to the stage or severity of the disease. His most striking results were in cases of amyloidosis in which 40 to 100 per cent of the dye disappeared in one hour. He noted such a disappearance only when

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there were extensive deposits of amyloid in the body, particularly in the liver. He found definite intravital staining of the amyloid tissue at necropsy in cases in which the dye had been injected as long as seven weeks before death, and later his experiments indicated that amyloid tissue had a definitely greater adsorptive affinity for Congo-red than does serum albumin. He also observed that in cases of chronic lipoid nephrosis the dye disappeared from the blood more rapidly than in normal subjects (40 to 60 per cent) and that in these cases the urine voided one hour after the injection was distinctly red and became blue with the addition of hydrochloric acid. This abnormal excretion of dye in the urine was found in seven cases of nephrosis and also in a few cases of nephritis and renal amyloidosis; the dye was not found in the edema fluid of cases of nephrosis.

Strasser confirmed Bennhold's observations in nephrosis, and found the dye in ascitic and pleural fluids which Bennhold had not been able to do. The latter observations have not been confirmed.

Schonberger and Rosenblatt described a case of albuminuria in which Congo-red was not excreted in the urine, and one case in which there was only a trace of albumin in the urine in which Congo-red was excreted. They also described three cases of nephrosis in which the dye was found in the urine and two cases of nephritis in which it was not found.

In this country Bookman and Rosenthal described one case of nephrosis with disappearance of 54 per cent of the dye in one hour and definite excretion of the dye in the urine.

DISAPPEARANCE OF DYE FROM THE BLOOD

We have studied the rate of disappearance of Congo-red from the blood in fifty cases, using the method described by Bennhold, except that we injected larger amounts of the dye, namely, 18 c.c. of a 1.5 per cent solution. This is the same dosage as was used by Rowntree and Brown for the determination of blood volume. The solutions were freshly prepared and care was taken that the dye was completely dissolved. Samples of blood were withdrawn three and sixty minutes after the intravenous injection of the dye, and the serum was compared colorimetrically with a standard (1:200 dilution) of the solution injected. The results given in Table I in general are confirmatory of Bennhold's observations.

TABLE I

RATE OF DISAPPEARANCE OF CONGO-RED FROM THE BLOOD

		DISAPPEARANCE OF DYE IN ONE HOUR, PER CENT		
	CASES	LOW	HIGH	AVERAGE
Miscellaneous diseases	27	10	36	22
Hepatic disease	10	11	39	17
Chronic lipoid nephrosis	9	19	41	30
Amyloidosis	4	75	100	89

We agree with Bennhold that although the average rate of disappearance of dye in hepatic disease is lower, and in chronic lipoid nephrosis higher, than in other diseases (except in amyloidosis), the limits of variability are too great to make the test of any diagnostic value for these conditions.

We have done the Congo-red test in four cases in which there was a disappearance rate of more than 75 per cent in one hour. Abstracts of the histories will be given.

Case 1 came to necropsy five days after the test was done, at which time the diagnosis of amyloidosis was confirmed and there was still definite vital staining of the amyloid tissue. This case has been reported elsewhere by Bannick and Barker. Cases 2 and 3 were clinically cases of amyloidosis. Case 4 had no clinical evidence of amyloidosis except an atypical renal lesion.

REPORT OF CASES

CASE 1.—A Mexican, aged thirty-seven years, came to The Mayo Clinic in 1928 with enlarged cervical lymph nodes. Biopsy of one of these nodes revealed lymphosarcoma of the Hodgkin's type. Extensive roentgen-ray therapy was given and the enlargement of the nodes disappeared. The patient returned a year later because of increasing weakness, vomiting, diarrhea, bloody stools, and abdominal pain for the last month.

Examination disclosed absence of enlarged superficial lymph nodes or enlargement of the liver or spleen. Albumin, graded 4, and a few erythrocytes were found in the urine. The return of phenolsulphonephthalein was 15 per cent in two hours. The blood urea was 58 mg., serum proteins were 3.8 mg., and blood cholesterol 235 mg. for each 100 c.c. There was gross blood in the stools. The systolic blood pressure was 90 and the diastolic 60; there were no demonstrable cardiovascular changes. The Congo-red test showed 75 per cent disappearance of the dye from the blood stream in one hour and dye, graded 1, in the urine in one hour. The test of hepatic function, by the bromsulphthalein method, showed retention of dye, graded 3.

The clinical diagnosis was previous lymphosarcoma of the Hodgkin's type, and renal and hepatic amyloidosis. The diagnosis at necropsy was residuum of lymphosarcoma of the Hodgkin's type in the liver (small nodule), and pancreatic and abdominal lymph nodes, and diffuse amyloidosis of the spleen, kidneys, liver, suprarenal glands and colon.

CASE 2.—A boy, aged twelve years, was brought to the clinic because of chronic empyema of the left side of the thorax for two years which had followed pneumonia and for which multiple operations for drainage had been done. Recently he had had attacks of vomiting.

Examination disclosed six discharging sinuses of the left wall of the thorax communicating with a large empyema cavity on the left side. Biopsy from one of these sinuses revealed tuberculosis. The liver and spleen were markedly enlarged. There was moderate secondary anemia. Urinalysis was negative and the blood urea was 15 mg. in each 100 c.c. By the Congo-red test there was 100 per cent disappearance of dye from the blood stream in one hour with no dye in the urine at the end of this time. The clinical diagnosis was chronic tuberculous empyema, and amyloidosis of the liver and spleen.

CASE 3.—A boy, aged thirteen years, was brought to the clinic with septic arthritis of the left hip for which surgical drainage, and later osteotomy, had been done. These procedures were followed by persistent draining sinuses. The condition had existed for three years. Attacks of vomiting had occurred recently.

Two draining sinuses of the left hip were found. Biopsy of one sinus disclosed inflammatory tissue. The hip joint was ankylosed, and roentgenographic examination disclosed old destructive arthritis. The liver was enlarged and the right kidney was palpable. There was moderate secondary anemia. The urine contained albumin, graded 3, erythrocytes graded 1, and hyaline casts graded 2. The blood urea was 33 mg. in each 100 c.c. With the Congo-red test 100 per cent dye disappeared from the blood stream, and a trace appeared in the urine at the end of one hour. The test of hepatic function by the bromsulphthalein method showed retention of dye graded 2. The clinical diagnosis was chronic septic arthritis of the hip, and amyloidosis of the liver and kidneys.

CASE 4.—A physician, aged fifty-seven years, had had progressive arthritis of a mixed infectious and senescent type for four years, involving first the shoulders, and then the elbows, hands, hips, knees, and ankles. A diagnosis of diabetes mellitus had been made three years before. Two years previously he had taken cinchophen hydroiodide (oxyliodide) 30 grains a day, for two months, and painless hematuria developed which stopped when he discontinued the drug. Weakness, loss of weight, and albuminuria had been noted for the last ten months.

Examination disclosed limitation of motion, tenderness, and hypertrophic changes in the shoulders, elbows, wrists, fingers, hips, knees, and ankles. There was slight edema of the ankles. Urinalysis showed albumin graded 3, erythrocytes graded 1, casts graded 1, and pus graded 1. The blood urea was 23 mg. in each 100 c.c. The return of phenolsulphonephthalein was 65 per cent in two hours. The maximal specific gravity of the urine was 1.017 and the minimal, 1.001. The hemoglobin was 15.8 gm. in each 100 c.c. Erythrocytes numbered 6,000,000, by the hematocrit there were 43 per cent cells, and blood volume (Congo-red) was 139 c.c. for each kilogram of body weight. Because of the condition of the kidneys, and the disproportionately high blood volume, a Congo-red test was done. Eighty per cent of dye disappeared from the blood stream in one hour; there was a trace of dye in the urine at the end of one hour. The hepatic function by the bromsulphthalein test showed dye retention graded 1. The blood volume (vital red) was 108 c.c. for each kilogram of body weight. The diagnosis was chronic infectious and senescent polyarthritis, amyloidosis of liver, kidneys, and probably of the spleen, and mild polycythemia.

This case illustrates a possible source of error in the determination of volume of blood and plasma by the Congo-red method. In cases of amyloidosis, when the blood is withdrawn at the end of three to six minutes (supposedly the time of maximal concentration) some of the dye has already been adsorbed by the amyloid tissue; therefore, the concentration in the blood plasma is less and a falsely high plasma volume is obtained just as it was in this case. We suggest that a high blood volume as measured by the Congo-red method, which is out of proportion to the values for the hematocrit readings, the hemoglobin, and the erythrocyte count, be viewed with suspicion. The Congo-red test should be done to determine the extent of disappearance of dye in one hour, and also the blood volume should be checked with vital red which is not adsorbed by amyloid tissue to any considerable degree.

These results tend to confirm the work of Bennhold and of previous investigators, in regard to the specificity of this test for amyloidosis, and one may consider that disappearance of more than 60 per cent of the dye from the blood stream in one hour is indicative of extensive amyloidosis. The test stands as the only laboratory test for the clinical recognition of this disease at the present time.

EXCRETION IN THE URINE OF CONGO-RED

Because of the fact that in certain forms of renal disease Congo-red was found by Bennhold and other observers to be excreted in the urine in quantities sufficient definitely to color the urine red, we have made observations on the excretion of this dye in the urine of 100 patients in an effort to determine what the factors are which permit such excretion, and in the hope of providing additional evidence as to the nature of the renal lesion. Observations were made as follows: 18 c.c. of a 1.5 per cent solution of Congo-red was injected intravenously. Voided specimens of urine were obtained at the time of the injection and one hour later. The second specimen was tested

for albumin with Exton's reagent and the grade of albumin was designated (Table II) as follows: no precipitate, 0; faint cloud, 1; translucent solution, 2; opaque solution, 3; and jelly-like solution, 4.

TABLE II

ALBUMINURIA AND EXCRETION OF CONGO-RED IN THE URINE

ALBUMINURIA, GRADE	CASES	CONGO-RED	
		GRADED 0 TO TRACE, CASES	GRADED 1 TO 2, CASES
0 to 1	45	45	
2	19	15	4
3	29	13	16
4	7	2	5

The one-hour specimen of urine was examined for Congo-red as follows: Two samples were placed in test tubes; a few drops of concentrated hydrochloric acid were added to one tube and the two tubes were compared. The amount of Congo-red present was graded as follows: In urine of normal color both without and with hydrochloric acid, graded 0; in urine of normal color which became slightly but definitely blue on the addition of hydrochloric acid, trace; in urine visibly red which became definitely blue on the addition of hydrochloric acid, graded 1, and in deep red urine which became deep blue on the addition of hydrochloric acid, graded 2.

We confirmed Bennhold's observation that in specimens of urine voided by most patients one hour after the injection of Congo-red, even if dye was not demonstrable by the addition of hydrochloric acid, there were traces which could be detected as definitely red on ordinary filter paper through which the urine had been filtered. In our series the largest amount of Congo-red excreted in the urine at the end of one hour, as determined by colorimetric methods, was 2 per cent of the amount injected. This was in a case of chronic lipoid nephrosis. Thus, the amount excreted in the urine is always too small materially to affect the disappearance rate in the blood stream, and cannot account for the fact that the disappearance rates are usually slightly but definitely increased in chronic lipoid nephrosis.

In the entire series of cases more than a trace of Congo-red in the urine was found only in cases in which there was albuminuria, graded 2 or more. The correlation of excretion of Congo-red and albuminuria is given in Table II. It may be noted that the dye was not excreted in all the cases of definite albuminuria, and therefore the presence of albuminuria does not necessarily imply that Congo-red will be excreted. Traces of Congo-red in the urine detectable only by the addition of acid were also found only if albuminuria was graded 2 or more, with two exceptions; a case of anasarca, probably of nutritional origin, in which albuminuria was absent, and a case of probable gouty kidney in which albuminuria was graded 1. At first it seemed that these traces of Congo-red in the urine might be significant, but on reviewing the data we have not been able to attach any significance to them and therefore include the cases in which only traces of the dye or none at all were excreted in one group.

The forty-five cases of various diseases without albuminuria in which dye was not grossly visible in the urine afford evidence that the normal kidney excretes the dye in small traces only. There were three cases of albuminuria in which the diagnosis of the renal lesion was indeterminate. The other fifty-two cases of albuminuria are listed with their diagnoses in Table III.

TABLE III

EXCRETION OF CONGO-RED IN ONE HOUR IN THE URINE IN DISEASES WITH ALBUMINURIA

	GRADED 2, CASES	GRADED 1, CASES	GRADED 0 TO TRACE, CASES
Chronic lipoid nephrosis	14		
Chronic nephrosis without edema	1		
Subacute and chronic glomerulonephritis with edema		6	3
Nephrosclerosis with albuminuria (malignant hypertension)		2	1
Renal amyloidosis		1	3
Syphilitic nephrosis with edema			1
Acute glomerulonephritis with edema			1
Chronic glomerulonephritis without edema			5
Residual albuminuria of acute glomerulonephritis			2
Renal chronic passive congestion			5
Hepatic cirrhosis with albuminuria			4
Diabetes mellitus with albuminuria			2
Bence-Jones proteinuria			1

The cases of chronic lipoid nephrosis are noteworthy since in all the dye was excreted in relatively large amounts. In the case designated chronic nephrosis without edema, the following was noted: high-grade chronic albuminuria with high blood cholesterol, low serum proteins, no cardiovascular changes, no hematuria and no history of edema. It is well known that in the edematous type of glomerulonephritis there are many of the clinical features common to cases of lipoid nephrosis. In the two cases of nephrosclerosis in which dye was definitely excreted, high-grade albuminuria of long standing without renal insufficiency had been present. At necropsy in the case of amyloid nephrosis in which dye was definitely excreted, extensive tubular degeneration of the kidney was found, as well as amyloid deposits in the glomeruli and arterioles.

In cases of chronic lipoid nephrosis, in addition to high-grade albuminuria, the two outstanding phenomena are alterations in the chemistry of the blood, namely, lowering of the serum proteins and increase in the cholesterol, and generalized edema. Whereas in most cases in which the excretion of Congo-red in the urine is graded 1 and 2 the serum protein is less than 5 mg. in each 100 c.c. and cholesterol more than 200 mg., these changes in the blood cannot be considered the essential factors, since in many cases in which Congo-red is excreted normal values for serum protein and blood cholesterol are obtained (Table IV). Changes in these factors may be merely concomitant in cases in which the condition responsible for the excretion of dye exists. It was Bennhold's belief that the altered serum proteins in lipoid nephrosis created a looser adsorption union of protein and dye and that this explained the usual more rapid elimination of the dye through the liver and also the excretion of the dye in the urine. In our cases the rate of elimina-

tion of dye from the blood in chronic lipoid nephrosis and also the comparison of serum proteins and excretion of dye in the urine are too variable to make this explanation adequate. However, it is possible that alteration in the concentration and character of the blood protein may assist in the excretion of the dye through an abnormally permeable kidney. Also, the incidence of edema, although suggestive, is not too significant (Tables V and VI). Apparently the presence or absence of edema in a given case at a given time has no influence on the excretion of Congo-red, but the factors which produce the edema or set the stage for the development of edema may also be concerned with the abnormal excretion of the dye.

TABLE IV

COMPARISON OF SERUM PROTEIN, BLOOD CHOLESTEROL AND EXCRETION OF CONGO-RED

	CONGO-RED	
	GRADED 0 TO TRACE, CASES	GRADED 1 TO 2, CASES
Serum proteins more than 5 gm. in each 100 c.c.	28	6
Serum proteins less than 5 gm. in each 100 c.c.	5	17
Cholesterol more than 200 mg. in each 100 c.c.	14	4
Cholesterol less than 200 mg. in each 100 c.c.	9	19

TABLE V

RENAL TYPE OF EDEMA AND EXCRETION OF CONGO-RED

	CONGO-RED	
	GRADED 0 TO TRACE, CASES	GRADED 1 TO 2, CASES
Edema slight or absent	56	6
Edema definite (graded 2 or more)	7	19

TABLE VI

CASES IN WHICH TESTS OF CONGO-RED WERE REPEATED AT VARIOUS STAGES OF EDEMA

	EDEMA, GRADE	CONGO-RED, GRADE
Chronic lipoid nephrosis	3	2
	2	2
	1	2
Chronic lipoid nephrosis	3	2
	0	2
Syphilitic nephrosis	2	Trace
	0	Trace

There are many unknown factors in the pathologic physiology of renal diseases. We offer the results of these tests of excretion of Congo-red as further data in such conditions. Undoubtedly albuminuria is associated with many different renal lesions of which inflammatory processes, destruction and degeneration of cells, and abnormal selective permeability of the epithelium are the most significant. Changes in the chemistry and osmotic pressure of the blood may also contribute. One or a number of these factors may be present in a given case in varying degrees. Although we lack definite proof, there is strong presumptive evidence that Congo-red is excreted either adsorbed to the molecules of serum albumin, or for the same reasons that the serum albumin is excreted. It cannot be a question of renal permeability to

molecules of a certain size for in that case if a molecule as large as that of Bence-Jones protein passes easily from the blood stream to the urine, a relatively much smaller molecule like that of Congo-red should also easily pass through the kidney. It is possible that there are certain types of albumin-permeable kidneys, exemplified by chronic lipoid nephrosis, which excrete both albumin and Congo-red, and others, exemplified by chronic passive congestion, which excrete albumin alone, a condition of selective permeability.

As far as we know, chronic lipoid nephrosis, which is the outstanding pathologic condition permitting Congo-red to be excreted in the urine in grossly visible amounts, has not been produced experimentally in animals. Assuming that the excretion of the dye was dependent either on a specific type of degenerative renal lesion which made the kidney abnormally permeable to certain substances, such as albumin, and therefore to the dye adsorbed

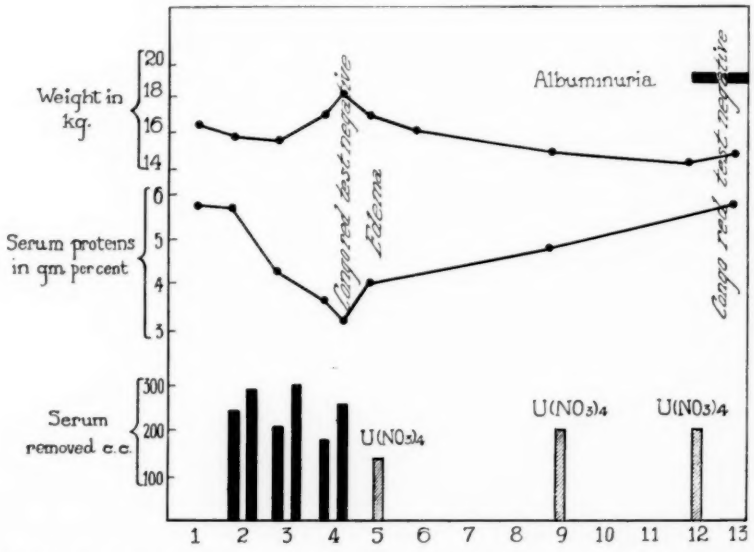

Fig. 1.—Effect on a dog of plasmapheresis and later renal injury by uranium nitrate, with failure to produce excretion of Congo-red in the urine.

to the serum albumin, or that the dye was carried through the kidney by abnormal types of protein in the blood stream, we attempted to produce such conditions in animals; the results were negative. Animals in which the kidneys were injured with uranium nitrate and with repeated injections of foreign protein did not excrete more than a trace of the dye in spite of definite albuminuria. Animals with experimental edema produced by plasmapheresis did not excrete the dye even with serum proteins as low as 3 gm. in each 100 c.c. (Fig. 1). Experimental animals given mixtures of egg albumin and Congo-red, whole horse serum and Congo-red, horse albumin and Congo-red, and horse globulin and Congo-red, intravenously, excreted albumin but not the dye. One animal given intravenously a mixture of Congo-red and the protein isolated from the urine of a patient with nephrosis excreted small amounts of urine definitely colored with the dye but this result was not obtained again after the experiment was repeated on several other animals.

This negative evidence tends to support the theory that the reason for excretion of Congo-red in chronic lipoid nephrosis and some allied conditions lies in the peculiar abnormal permeability of the kidneys for protein in these diseases. We are forced to conclude that this must be a different type of permeability than exists in other conditions with albuminuria, such as chronic passive congestion and the dry types of glomerulonephritis. Possibly specific abnormal types of serum protein have some influence on this type of permeability.

SUMMARY

Bennhold's Congo-red test as a specific clinical test for amyloidosis has been confirmed by us in four cases with forty-six controls.

In the absence of albuminuria Congo-red is excreted in the urine in traces or not at all.

In fourteen cases of chronic lipoid nephrosis Congo-red was excreted in the urine in relatively large amounts.

In some cases of glomerulonephritis with edema, nephrosclerosis with albuminuria, and renal amyloidosis, Congo-red was excreted in the urine.

In cases of nonedematous glomerulonephritis and chronic passive congestion of the kidneys with albuminuria, only traces, or none at all, of Congo-red were excreted in the urine.

Cases in which definite amounts of Congo-red were excreted in the urine were usually those with (1) albuminuria of high degree; (2) serum proteins below 5 gm. in each 100 c.c.; (3) blood cholesterol above 200 mg. in each 100 c.c., and (4) edema of the renal type.

Efforts to produce excretion of Congo-red in the urine in animals were not successful.

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SURFACE HEATING WITH TWO TYPES OF DIATHERMY ELECTRODES*

By A. Hemingway, Ph.D., Minneapolis, Minn.

IN THE heating of the body tissues by diathermy currents the important clinical problem is the proper dosage. With the machines available at present the dosage is measured by the current passing between the electrodes, the value of the current being read from a high frequency millimeter in series with the patient. The current is adjusted to give 10 to 20 milliamperes per square centimeter of the smallest electrode, according to Burmeister[1] while Corbus and O'Connor[2] recommend 100 milliamperes per square inch.

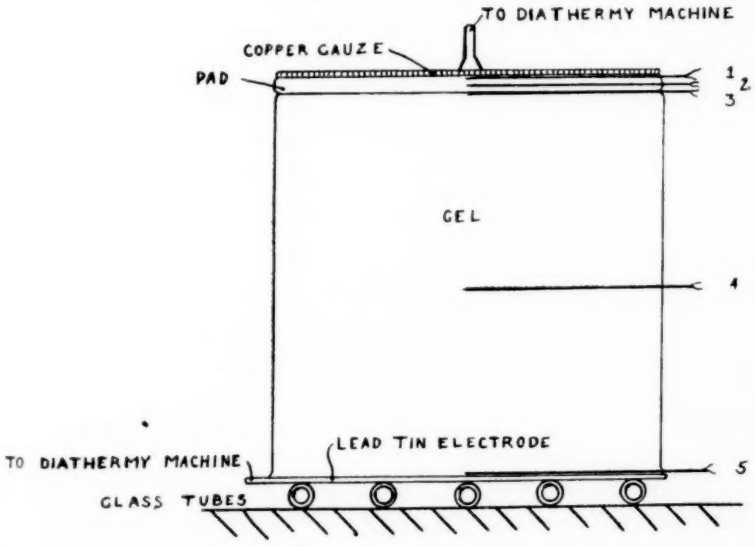

Fig. 1.

Two types of electrodes are in common use. One type is a pliable electrode of sheet lead and tin and the other type a cotton pad soaked in saline with one side of the moist pad pressed to the body and the other side of the pad covered with copper gauze which is connected to the diathermy machine. Recently clinicians have observed a greater heating effect from the metallic electrode than from the moist pad electrode for the same current dosage. Bordier[3] placed a pad electrode of 20×22 sq. cm. on the epigastric electrode and a large indifferent metallic electrode on the lumbosacral region. After forty minutes with a current of 5000 milliamperes passing between the electrodes no physiologic heating effects were observed, i.e., no perspiration, no

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quickening of the pulse rate nor redness of the skin under the electrode. On replacing the saline pad electrode with one of the same area of pliable tin, after twenty minutes' treatment with 3000 milliamperes he noted free perspiration, a rapid pulse and accelerated respiratory movements. Simon,[4] using a mercury thermometer under the skin of a patient locally anesthetized with novocaine and adrenalin has observed a slight increase of temperature before the surface of the skin for a metallic electrode over that of a pad electrode.

It is with a purpose of determining if the diathermy dosage should be varied with the type of electrode that the following experiments have been performed. It has been our purpose also to investigate in a quantitative manner the cause of this variation.

A saline agar gel of approximately 60 ohms high frequency resistance was used as a model to study the heat distribution. This resistance as we have

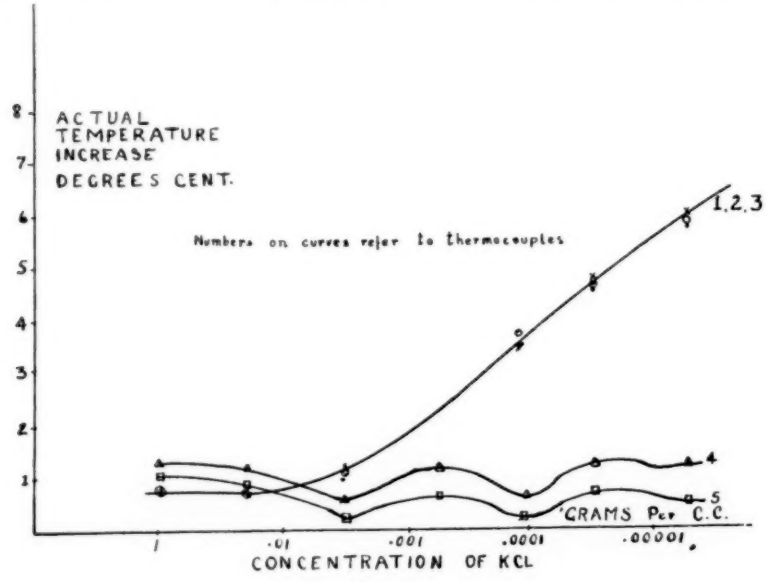

Fig. 2.

shown previously[5] represents the average value of the resistance of the patients to the diathermy current. The gel was cylindrical in shape and one end rested on a metallic lead-tin electrode which in turn was supported on glass rods allowing circulation of the air below the electrode. The saline pad electrode (6 mm. thick) was placed on the upper end of the gel and pressed to it by a lead ring which rested on the copper gauze. Thermocouples were placed in the gel and arranged as follows (see Fig. 1):

Thermocouple (1) between Cu. gauze and saline pad
" (2) in center of saline pad
" (3) between pad and gel
" (4) in center of gel
" (5) between metallic electrode and gel

The thermocouples were connected to double throw switches and a low resistance galvanometer giving a deflection of 8.4 cm. per degree. Each thermo-

couple was made from a No. 38 wire and each wire in the gel was surrounded by a thin-walled capillary glass tube. The junction outside the ends of the glass tubes was covered with collodion.

For resistance measurements of the gel and pad a high frequency whetstone bridge was used (for details see McClendon[6]). Resistance measurements were made with an A. C. current of 10^6 cycles per second. Measurements were taken before and after the gel was heated by the diathermy current and the average value of the resistance computed.

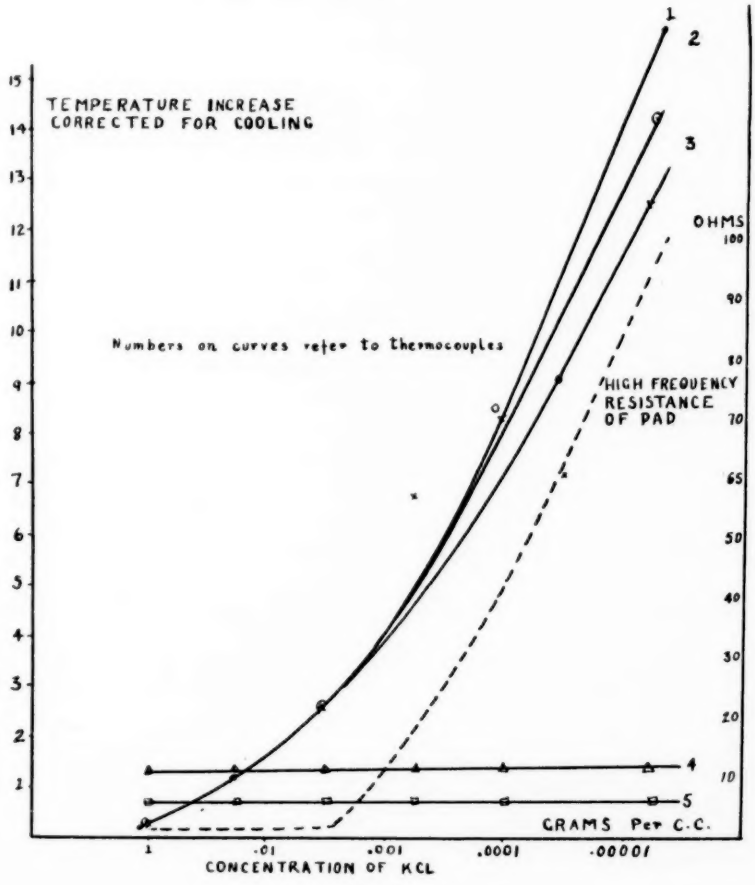

Fig. 3.

The gel was allowed to stand in a room at 24° to 25° until thermal equilibrium was reached which lasted several hours. A current of 0.250 amperes was passed through the gel from a Victor diathermy machine for ten minutes. The temperature increase was noted and the results plotted on large graph paper, temperature being plotted against time. On the same paper radiation curves were plotted, that is the amount of cooling was measured and a correction made for the heat lost due to radiation or cooling (for details see [7]). By this method the true temperature increase was computed assuming no loss of heat due to any sort of cooling.

The pad was soaked in KCl solutions of different concentrations to study the effect of the concentration of the electrolyte in the pad on the heat distribution. Time-temperature curves were drawn for the various pad electrodes containing the varying concentrations of KCl. The *actual* temperature increases as well as the *theoretical* temperature increases assuming no cooling were computed from the curves. These temperature increases for the pads containing the varying electrolyte concentrations, the resistance of the pad and the total resistance of pad and gel are given in the curves of Figs. 2 and 3. Fig. 2 gives the actual rise in temperature as measured by the thermocouples. On the abscissa are plotted the concentration of the KCl in the pad. In Fig. 3 the theoretical temperature increases assuming no cooling is given. From the ordinate at the right the values of the corresponding resistances may be obtained.

From these curves it is seen that the temperature increase in the gel depends very little on the concentration of electrolyte in the pad. The temperature increase at the metallic electrode is less than that inside the gel. For a concentrated saline electrode the surface heating is less than that at the metallic electrode. With a weak saline electrode, however, a greater increase of surface temperature occurs than at the metallic electrode.

The difference between the actual temperature rise and that corrected for radiation losses, indicates the importance of cooling due to evaporation from the moist electrode. This is illustrated more clearly in the time temperature curves, a sudden drop occurring at the point where the current is stopped.

The higher surface heating due to low concentration of electrolyte in the pad, noted also by Bordier and Simon, is shown by the curves to be a pure resistance effect. The lower the resistance of the saline pad due to a more concentrated electrolyte, the less is the surface temperature increased.

CONCLUSIONS

In order to avoid surface heating with saline electrodes, the pads must be soaked in salt solutions having a conductivity greater than 10 per cent KCl.

Evaporation which produces cooling at the saline pad lowers the surface temperature below that of the metallic electrode. Hence a larger current can be used with a saline pad electrode than with a metallic electrode.

In conclusion I wish to thank Dr. W. K. Stenstrom and Dr. J. F. McClendon for many helpful suggestions during the course of these experiments.

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LABORATORY METHODS

THE INTRADERMAL TEST AS AN AID IN THE DIAGNOSIS OF UNDULANT FEVER*

By William Levin, Dr. P.H., Portland, Ore.

THE laboratory diagnosis of undulant fever is usually carried out by blood culture, agglutination reaction, or animal inoculation. Of these three methods, the agglutination reaction is undoubtedly the most generally in use, since it is not only specific, but can also be easily and rapidly performed by any laboratory.

Just what constitutes a diagnostic agglutinin titer for undulant fever has been a debated subject. The majority of workers are agreed that a titer of 1-80 or above is diagnostic. Numerous cases, however, have been reported where the agglutinin content was far below this dilution, or could not even be demonstrated. Carpenter and Boak[1] report that in 6 per cent of their cases no agglutinins were present, and that they have observed severe cases of undulant fever with a maximum titer of 1-15 and 1-30. Nicolle, Burnet and Conseil[2] injected 5 adults subcutaneously with living cultures of Br. abortus and failed to demonstrate agglutinins in the serum of two following the injections. Giordano and Sensenich[3] report two active cases of undulant fever whose serum did not agglutinate the Brucella in a dilution higher than 1-10.

The intradermal test for undulant fever may be of aid in arriving at a diagnosis where either blood culture or agglutination, or both these tests, are negative. The earliest work on the intradermal test was done by Fleischner and Meyer[4] in 1918. They injected 30 guinea pigs with the sediment of certified milk, and later gave an intradermal injection of a 1-1000 specially prepared Br. abortus protein. All of the animals gave a positive skin test. In 1922 Burnet[5] introduced an intradermal test for undulant fever in which he used a filtrate (Chamberland) from a culture of killed organisms. Boiling for one minute did not destroy the activity of this filtrate, which he called "melitine" or "abortine." A drop of the filtrate placed upon the skin, and the skin then scarified gave no reaction. A positive reaction appeared from six to seven hours after the intradermal injection, and was characterized by local edema, accompanied by redness and even slight pain. The zone of the reaction was 4 to 6 cm. in diameter, with a regular and well-defined contour. The reaction persisted for several days. Burnet[6] states that his test has never given a negative result where other diagnostic procedures were positive, nor has he ever seen it give a positive reaction in other febrile diseases such as typhoid, malaria, or tuberculosis.

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Bastai and Rotta[6] report good results with Burnet's reaction, but they occasionally obtained positive reactions with patients believed to be uninfected. They admit, however, that these persons may have consumed milk contaminated with the Br. abortus. Giordano[7] cites several Italian workers who do not regard Burnet's test as satisfactory; he himself considers the test of insufficient specific value.

Giordano used a heat-killed saline suspension of Br. abortus and obtained severe local reactions by injecting it intracutaneously in twenty-five proved cases of undulant fever. One hundred controls similarly injected yielded negative reactions in 99 per cent. The positive control was in a suspected tuberculous person. The amount injected was 0.2 c.c. of a suspension having a silica turbidity standard of 1:1000. Giordano states that even 0.05 c.c. amounts yielded specific reactions.

Simpson and Frazier[8] obtained positive tests after the intradermal injection of 0.1 c.c. of a suspension of killed B. abortus organisms in ten cases whose anti-abortus agglutinins were present in titers ranging from 1:320 to 1:1280. In 25 patients suffering from a variety of other diseases, they obtained negative results.

The intradermal test for undulant fever was deemed of sufficient promise for further investigation. A suspension of Brucella abortus (strain No. 80) was prepared according to the method of Giordano, and 0.05 c.c. was given intradermally to thirteen persons at the State Tuberculosis Hospital, Salem. One of these was Dr. P. L. N., a recovered undulant fever patient. He and three others who had had anti-abortus agglutinins not only showed a marked local reaction, but also had a more or less severe general reaction. Six other patients with known anti-abortus agglutinins gave local reactions only. Three of the patients whose blood was negative for agglutinins gave no reaction. The local reactions were very marked and severe varying from ¾ of an inch to 4 inches in diameter, and in several producing granulomatous lesions with consequent discoloration of the injected area.

Because of the severity of the reactions an attempt was made to produce a less toxic antigen. The method used was a slight modification of that described by Fleischner and Meyer.[4] This method yields a protein that gives a distinct reaction, but one which causes no discomfort. None of the patients tested with this antigen gave a general reaction.

Brucella abortus, strain No. 80, was grown on liver infusion agar in Blake bottles, incubated for seventy-two hours at 37.5° C. and the growth washed off with sterile saline. The suspension of organisms was then centrifuged, the supernatant fluid removed, and the bacterial sediment resuspended in sterile saline. This was again centrifuged, and the sediment twice washed with 50 per cent alcohol, once with 95 per cent alcohol, and once with absolute alcohol. The washed bacterial cells were then extracted with absolute alcohol in a Soxhlet apparatus for two days, and then with ether for two days. After the final extraction the fat-free bacterial cells were dried and thoroughly ground in an agate mortar.

The standard antigen suspension was made up so that the skin test dose,

0.2 c.c., contained 0.000004 gm. ($\frac{1}{250}$ mg.) dry bacterial protein. Each lot of skin test antigen was tested for sterility. No preservative was used.

Through the kindness of many physicians a number of definitely diagnosed as well as suspected cases of undulant fever were tested intradermally with the abortus protein. Tests were also made on the patients at the Oregon State Tuberculosis Hospital, Salem, where an outbreak of undulant fever occurred in May, 1928;[9] on patients at the Eastern Oregon Tuberculosis Hospital, The Dalles; on patients at the Doernbecher Memorial Hospital for Children, Portland; and on many normal persons.

A positive skin reaction usually appears in from eight to twenty-four hours, being most marked at thirty-six to forty-eight hours after the injection. It is characterized by local redness, and induration, with a frequent central necrotic area, and persistency of reaction for several days. Not infrequently a discolored scar remains at the site of the injection. The extent of the zone of the reaction varies from 2 cm. to 8 cm. in diameter. A striking characteristic has been noted in that patients with low agglutinin content and a history of chronic infection gave the most severe reactions, while those in the acute stage and with a high serum agglutinin titer gave rather weak reactions. Contrary to the findings of Giordano[10] who reported an appreciable increase in agglutinin content in positive reactions and the appearance of agglutinins in 70 per cent of the negative reactors after the intradermal injection of his killed suspension of Brucella abortus, no such results were obtained with the fat-free abortus antigen.

Of fifteen clinical cases of undulant fever all gave positive intradermal tests. Table I illustrates the age and sex incidence as well as the serum agglutinin titers of the patients.

TABLE I

AGE GROUP	TOTAL NUMBER TESTED	SKIN TEST	AGGLUTINATION TITER								
			1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
Under 20	1	+	F								
20 to 30	1	+									M
30 to 40	8	+	F-M		F		2F-M	M		M	
40 to 50	3	+			F	M	M				
50 to 60	1	+		M							
60 over	1	+							F		

A study of Table I shows that the incidence of the disease was equally divided between the male and female sexes, and that the age group of thirty to forty included a little over half of the cases. Only one child is represented in this series, a girl nine years of age.

Fourteen patients with varying symptoms, but all giving histories of chills and sweats, and many pyrexias, gave negative intradermal reactions. With one exception all gave negative agglutination tests. A case later diagnosed influenza gave a positive agglutination test in 1/20 dilution. Three of the fourteen patients were subsequently diagnosed as having typhoid fever, and one lobar pneumonia.

Ninety-four patients at the Eastern Oregon State Tuberculosis Hospital were given the intradermal test by Dr. DeWalt Payne, the resident physi-

cian. Only one of these gave a reaction which might be interpreted as doubtful. Her agglutination test was negative. According to Dr. Payne, "this patient started drinking a large quantity of milk, about one and one-half quarts a day, following diagnosis of tuberculosis in January. She had averaged about one glass a day previous to that. The milk was obtained through various stores in Portland."

One hundred and sixty-nine patients at the State Tuberculosis Hospital, Salem, were given the intradermal test. Seven gave distinctly positive reactions. It is of interest to note that six of these reactors were patients at the hospital in May, 1928, when, as already stated, there occurred an outbreak of undulant fever. At that time the whole hospital population and staff yielded forty-three who gave agglutination reactions in titers varying from 1/20 to 1/2560, and five of these were reported as clinical cases. All six of the skin reactors had shown anti-abortus agglutinins in 1928, and three had some agglutinins at the time the intradermal test was made. This is illustrated in Table II.

TABLE II

PATIENT	AGE	SEX	SKIN TEST	AGGLUTINATION TITER	
				1928	1930
B. S.	8	M	+	1- 20	-
M. S.	44	F	+	1-120	1-10
A. J.	66	M	+	1-160	-
E. K.	27	M	+	1-160	1-10
J. W.	30	M	+	1-160	-
J. K.	66	M	+	1-160	1-20

Dr. G. C. Bellinger, Superintendent of the State Tuberculosis Hospital furnished the following information concerning these reactors, taken from clinical notes made from April 13, 1928, to July 15, 1928:

"B. S. was clear as having no symptoms and as not ill.

"M. S. was ill during the first part of this period, and then showed improvement. On July 15, we note: 'No temperature for two months, no suggestive symptoms. Lived on a farm when young. Infectious abortion among cattle in the neighborhood, but she claims that she never used milk at all while on the farm.'

"A. J. was on exercise during this period. No special illness. No temperature for months except for a day or two, and had no suggestive symptoms.

"E. K. had a high titer. Had lost seven pounds, was in good general condition; no fever for over a year and no suggestive symptoms whatever. His entire history was negative as to contact with possible infection.

"J. W. had a high titer; lost 2½ pounds when a bed patient, and had an intermittent fever during several months of this period; otherwise negative.

"J. K. had many tests, showing a variable titer over a period of two or three months under observation. He had had some fever on May 28, which was apparently due to a cold, with no suggestive symptoms. He had lived on a farm and milked cows for years."

No information is available concerning the other skin reactor, who also gave a negative agglutination test. Eight patients who had some agglutinin content in 1928 gave negative skin tests.

Twenty-one medical students were given the intradermal test. Two gave typically positive reactions. They also gave marked reactions when injected intradermally with the fat-free Brucella proteins made from strains 427, 428 and 456. Both students (aged twenty-one and twenty-two) were healthy, and gave no history of past undiagnosed illness. The serum of one agglutinated weakly (2+) Br. melitensis variety abortus, strain 456, in a dilution of 1-5. The agglutination reaction of the other student was negative. Both students had used raw milk exclusively up to about a year ago.

In the course of the routine physical examination of milk handlers by the Portland Health Bureau, a twenty-two-year-old lad was found whose serum agglutinated Br. abortus in a dilution of 1-160. He gave a positive intradermal reaction. Here again there was seemingly no history of past infection or undiagnosed illness, although he admitted having milked cows for the past five or six years.

A nine-year-old girl, D. W., with an undiagnosed illness of over one year's duration was given the intradermal test for undulant fever. She gave a very marked reaction. Her agglutination test was positive in a dilution of 1-40. Her history presented a classical picture of undulant fever infection. The milk used in this girl's home had always been pasteurized, and an attempt to link her infection with a visit to her grandfather's ranch six weeks prior to the onset of her illness failed when it was shown that no reactors had been found among the cattle at that ranch. It was found, however, that the girl visited a neighbor rather frequently and often drank milk there. This neighbor's child, a nine-year-old boy, D. P., also had had an undiagnosed illness of over a year's duration. He had been under observation at the Doernbecher Memorial Hospital for Children as a suspected case of tuberculosis. D. P. gave a very marked intradermal test for abortus. A tuberculin test given him at the hospital had been read as slightly positive. His serum, tested several times, failed to show any agglutinins for the Brucella. His mother, Mrs. P., also gave a marked intradermal reaction for abortus, and an agglutination titer of 1-160. She gave a history of having had what she thought was the "flu" six months prior to the test, and had been ill for several weeks with rheumatic pains. Both the mother and boy drank raw milk in quantity. The milk was produced at the S—Dairy. Through the kindness of the Milk Division of the Portland Health Bureau the herd was tested for contagious abortion. Out of 40 cows tested 12 were definite reactors and six were classified as suspicious.

Thirty-three children were given the intradermal test at the Doernbecher Memorial Hospital for Children. The ages ran from six months to fourteen years, with the majority being in the 4-8 year group. Only one child, M. N., a five and a half year-old girl, gave a positive reaction. Her agglutination test was negative. She also gave a negative tuberculin test. The clinical diagnosis on this patient was sinusitis and mastoiditis.

Fleischner and Meyer[4] reported that 75 infants who had been fed on a high bacillus abortus-containing milk gave, with two exceptions, negative intradermal tests. The exceptions were in two children, aged about two years, who were suffering from chronic bone tuberculosis. These two children also gave positive skin tests with typhoid protein. They concluded that B. abortus was not pathogenic for infants. Kohlbry,[11] however, reported a case of undulant fever in a year-old child with an agglutinin titer of 1/1280.

Seventeen persons, healthy adults, used as controls at various times, gave negative intradermal tests. On three of these, negative agglutination tests were also obtained.

SUMMARY

In all, 365 individuals were given the intradermal test for undulant fever. Twenty-seven gave distinctly positive reactions. Of these 15 were definitely proved clinical cases of undulant fever; all gave positive agglutination tests. One reactor, a nine-year-old boy, gave such a characteristic symptomatology that he may well be considered a clinical case in spite of his yielding negative agglutination tests. Six reactors had given positive agglutination tests nearly two years prior, and had been patients at a hospital at a time when there had occurred an outbreak of undulant fever. One reactor, with a positive agglutination test, was a healthy individual whose work as a milker had brought him in close contact with cattle. Four reactors gave negative agglutination tests and no history of undiagnosed illness.

What is the significance of the intradermal test for undulant fever? In so far as the experimental animal is concerned, Fleischner and Meyer[4] had already stated in 1918, that "cutaneous hypersensitiveness as evidenced by positive intradermal tests in guinea pigs is an absolutely positive sign of infection by the B. abortus bovinus. This fact is equally true whether the disease is acute or chronic in character." The inability to demonstrate anti-abortus agglutinins in certain human cases of undulant fever has been reported by trustworthy investigators, hence the absence of agglutinins in a reactor to the intradermal test is not conclusive evidence of absence of infection. The possibility of mild infections and carrier conditions with the Brucella is admitted by many workers. Thus Carpenter, Boak and Chapman[12] state: "Evidently the majority of strains of B. abortus are only slightly pathogenic for man, therefore it is easily conceived that an individual may have an invasion of the tissues by this organism resulting in the production of abortus agglutinins without causing any visible symptoms of the disease." Meyer and Eddie[13] conclude that "the constant ingestion of a small number of Brucella organisms in cow's milk during infancy may lead to an acquired immunity or to a carrier state."

Unfortunately the testing of an individual for an undulant fever carrier condition is extremely difficult in so far as the isolation of the organism is concerned. Attempts to isolate the organism from the stool and urine from undulant fever patients as well as from suspected carriers resulted in failures. The method described by Amoss and Poston[14] for stool culture was rigidly followed, but without success.

According to Fleischner and Meyer[4] "cutaneous hypersensitiveness is positive whether the Br. melitensis or the Br. abortus is used as antigen. If guinea pigs are infected with abortus bacilli, skin tests are as strongly positive to melitensin as to abortin, although all other bacterial proteins give negative results." Intradermal tests made on several guinea pigs and rabbits infected with Br. abortus or Br. melitensis gave rather inconclusive results when proteins made from the various strains of the Brucella were used interchangeably, although bacterial proteins other than the Brucella gave negative results. It is of interest to note that the filtered saline washings of a suspension of Br. abortus made off an agar culture elicited a reaction when injected intradermally in infected animals.[15] Schoenholz and Meyer[16] obtained intradermal reactions with a filtered cell solution of Br. abortus, as well as with a substance from this solution precipitated by acetic acid in the cold at P_H 3.5 to 4.0. The specificity of the cutaneous reaction will undoubtedly be enhanced by the use of a pure protein.

CONCLUSIONS

1. The intradermal test for undulant fever holds promise of being of distinct diagnostic aid to the clinician and merits his serious consideration and study.

2. In the absence of anti-abortus agglutinins a positive intradermal test may be diagnostic of undulant fever; hence the intradermal test should be performed on all individuals whose agglutination reactions are negative.

3. If in suspected chronic cases of undulant fever both negative intradermal and agglutination tests are obtained, infection with Br. abortus may be safely ruled out.

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A STUDY OF GLASS PARTICLES RESULTING FROM THE BREAKING OF GLASS AMPULES*

By Stanley O. Chambers, M.D., Los Angeles

AN INTERESTING observation and one of possible practical value was uncovered during the experimental study of "local reactions from intramuscular medication" and seemed of sufficient importance to warrant this contribution which represents the first of a series. This communication is confined to the study of glass particles resultant upon the breaking of glass ampules in preparing medicaments for administration. In view of the fact that the presence of glass particles in glass ampules after breaking of the tip has long been suspected, it is remarkable that the aspiration of these particles through the needle and into the syringe and their subsequent injection into the tissues has only once been suggested as possible and never before demonstrated.

If the method of filtration through sterile cotton or gauze were universally accepted and used, the practical value of this demonstration would indeed be limited in its application. I am reasonably sure, however, that most drugs are prepared for intramuscular administration by utilizing the ampule as a container where possible for mixing and dissolving, followed by withdrawal of the solution into the syringe without filtration through sterile cotton. It is likewise true that this latter step of filtration is usually omitted when removing solutions from containers other than the ampule. Solutions sealed in ampules are always aspirated directly into the syringe. Oil suspensions are emptied directly from the ampule into the syringe without filtration, for oil suspensions do not adapt themselves to filtration.

The presence of these glass particles as foreign bodies in the ampule or container is not of itself important. Their possible aspiration through the needle and into the syringe is, for it is reasonable to suppose that such particles could be as easily expelled as aspirated. In proof of this supposition, the following experimental studies are offered. No attempt is made to show that these particles are in any way related to the local reactions so frequently met with in intramuscular medication, especially with those drugs so commonly used in the treatment of syphilis, nor to question their possible harmful effect in the tissues. The fact that a foreign element (glass) is present in medicaments and is unintentionally being injected into tissues seems of sufficient interest.

The mechanics involved in the deposition of glass particles into the bottom of a glass ampule are simple of explanation. A glass ampule is filed at its neck, pressed by the fingers (a sterile towel being placed between the

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ampule and the fingers) and the tip broken off; the process of breaking results in fine particles of glass falling to the bottom of the ampule.

It is obvious that the amounts are not constant but depend on the grade and thickness of the glass and the mechanics of breaking. Friction against the broken edges of the ampule by the needle while dissolving the contents likewise results in glass particles being dislodged and deposited in the bottom of the ampule. Glass particles in varying amounts were found in the bottom of all ampules studied. This fact has been mentioned and a method of filtration through sterile cotton suggested to prevent the theoretical aspiration of these suspected particles into the syringe.

Glass drops to the bottom of a solution and if not agitated remains there. A large part of the ampule's contents theoretically therefore could be aspirated free from glass (care being taken to keep the needle tip just below the surface and the solution at rest). The aspiration of glass particles however is possible regardless of care in removal, or of the amount removed, when agitation is a factor. The amount of a solution free from glass particles which can be removed from an ampule has been roughly determined.

Granted that it is possible to remove from an ampule or container a large part of the solution free from glass, that amount would not represent the dosage as contained in the ampule, for the entire solution could not be removed. Hence, it would be necessary to dissolve more of a given drug than needed in order to obtain an amount equivalent to the desired dose. This suggestion might be applicable to aqueous solutions but certainly not to oil suspensions, for here the glass particles are also in suspension.

In this study glass particles were demonstrated in varying amounts regardless of the type of ampule studied. For experimental purposes glass ampules of approximately 3.5 to 4 gm. in weight and ¾ of a mm. in thickness were used. The glass, according to the manufacturers, is termed a good bacteriologic grade. The knowledge that certain grades of glass contain impurities capable of tissue irritation has been kept clearly in mind.

An attempt was first made to demonstrate the glass particles resultant upon breaking of the ampule tip. For this purpose the bottom of the ampule was filed and broken off, the edges smoothed by flaming, its contents removed and the ampule thoroughly cleaned and dried. The tip was then filed and by pressure of the fingers, broken off, whereupon a veritable shower of glass particles could be seen. The open bottom was carefully held over a clean glass plate; examination of the plate under low power magnification revealed many particles of glass of variable size and shape. A total of fifty ampules were then broken and an approximate count of the particles made for each ampule. The greatest number counted for any one ampule was approximately 500, the smallest being approximately 200, while an average of 320 was attained for each ampule.

Utilizing these same ampules, i.e., with open bottoms and broken off tips, an intramuscular needle of twenty gauge was allowed to rub gently against the broken inside edges of the ampules, care being taken to hold the open bottom over a glass plate as before. Upon examination of the plate with

the low power objective, glass particles were evident, dependent in numbers upon the amount of friction.

The second method used for this demonstration consisted in filing the tip of an empty intact glass ampule and breaking it as before. The bottom of the ampule was then examined with the lens; glass particles could be seen in relatively large amounts in the bottom of the ampule and in all probability exceeded in amount the former method, since the vacuum in the ampule was not disturbed as was before necessary.

Into each ampule (50), broken as above, 0.5 c.c. of sterile water (triple distilled) was injected. By aspiration through a 20 gauge, 1½ inch steel needle, the entire 0.5 c.c. was removed into a 1 c.c. syringe. In most cases glass particles could be seen in the dependent portion of the syringe upon examination with the lens. The contents of the syringe, 0.1 c.c. at a time,

Fig. 1.—Microphotograph showing glass particles as expelled through a 20-gauge intramuscular steel needle. Low power objective.

were placed upon clean glass slides and examined with the low power objective; a cover slip was placed over this amount of solution before examination. The first 0.1 c.c. expelled, revealed an average of 65 per cent of the total particles, the second 0.1 c.c. revealed 25 per cent, the third, 10 per cent. No particles were seen in the remainder. The syringe in each case was kept at rest with the needle tip in a dependent position throughout. (Fig. 1.) Controls were run using sterile distilled water (flask) with identical syringes and needles. All controls were negative.

The foregoing evidence was sufficiently convincing to show that glass particles can be easily aspirated from an ampule through the needle and into the syringe and in turn expelled. Other needles, ranging in size and bore from hypodermic to gauge 18, were studied and glass particles were found to pass through all, in amounts depending upon their size.

Obviously, if the entire fluid content of an ampule is not removed, less glass is aspirated. The exercising of such care in the position of the needle tip while aspirating proved to be difficult, especially when dealing with such small amounts of solution, although it was possible to remove 0.3 c.c. of a total of 0.5 c.c. of the solution, free from glass. The remaining 0.2 c.c. invariably showed particles on examination. This fact if constant would be of considerable assistance providing glass ampules were in some way graduated. One could then remove all but the last 0.2 c.c. and be questionably certain that no glass had been aspirated. Unfortunately, agitation of the ampule contents while aspirating makes this observation exceedingly inconstant, for upon marked agitation glass particles can be found after removal of the first 0.1 c.c. This difficulty is in part due to the small amount of the solution. Practically, this is the case with many of the intramuscular medicaments which we are now using, the amounts in some cases being little more than 0.5 c.c. Where the amount of the solution is greater, naturally more of it can be aspirated without containing glass. The removal of such a solution, however, whether large or small amount, is at best little more than a gamble.

Ampules of oil suspension were next examined after the previous method, i.e., breaking the tip, emptying the contents into a syringe and then expelling it through a 20 gauge needle drop by drop on glass slides. Here again glass particles were consistently demonstrated as being present in the ejected material, although the amount of the suspension, its oily character and the probable greater dispersion of the particles rendered identification less easy, there being fewer to each microscopic field.

The derivation of the glass particles, i.e., inner or outer surface of the ampule, presented a rather pregnant question, as the sterilization of the average ampule before use is not always complete and thorough. The inner surface of glass ampules is considered sterile at the time of sealing by the manufacturer, while the outer surface must be sterilized by immersion in alcohol for fifteen minutes before use. Failure to adhere to the latter rule, with the possibility that some of the glass particles came from the outer surface of the ampule, makes for possible contamination. Cultural studies of the particles under various sterilization methods are being investigated at the present time.

It seemed desirable to demonstrate the derivation of the particles, hence the outer surface of a series of empty intact ampules were immersed in eosin which effectually coated them. The ampule tips were filed and broken, and the glass as before dropped to the bottom. A few drops of an alkaline solution placed in the bottom of the ampule in contact with the particles resulted in a pink colored solution showing in all probability that some of the dislodged particles were derived from the outer surface of the ampule; such a positive demonstration was obtained in only one out of one hundred attempts.

CONCLUSIONS

1. Glass particles from opening of glass ampules contaminate the contained medicament.

2. They can be withdrawn from the ampule through the needle and into the syringe.

3. They can be expelled from the syringe through the needle.

4. In one attempt out of one hundred, particles were shown to be from the outer surface of the ampule.

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A FECEOMETER OF SIMPLE CONSTRUCTION*

By Raymond J. Miller, B.Sc., M.Sc., Ph.D., State College, Pa.

RECENT investigations concerning intestinal absorption and rate of defecation in the rat as influenced by varying dietary conditions indicate the importance of data relative to the normal rate of defecation when using this animal as an experimental subject. The general method of abdominal manipulation to aid intestinal evacuation appeared too original and also faulty in that the general tonicity of the intestinal tract is likely to be altered beyond that as influenced by the ration under consideration. It appeared that any apparatus which will record the natural rate of defecation and the collection of feces would be of considerable value. Such an instrument has been devised by the author and, at present, is meeting with considerable success in our animal laboratory.

Fig. 1 illustrates the feceometer in complete assembly and operation. The apparatus consisted primarily of two open-frame adjustable shelves for cage and instrument mounting which were supported by two iron laboratory stands. Accessory instruments such as the trip-pan lever, electric recorder, etc., were also attached to these uprights.

The upper shelf supported an animal cage with an attached funnel duly housed. Both cage and funnel were made from one-half inch mesh hardware cloth and each measured ten inches in diameter and eight inches deep. A second funnel made from fly screening was inserted into the one of hardware cloth and securely held in place. A small metal funnel having a half-inch neck and about four inches long (Fig. 2) was attached to the larger cloth one. It will be noticed that a window was made in its neck for the insertion of the trip-lever pan which is described later.

The lower shelf was also of open construction and supported a simple kymograph, a feces-collecting wheel and dry cells.

A simple kymograph was made from a Big Ben clock, a two-quart Karo container, and three-eighths inch steel or brass rod. After pointing one end of the rod and cupping the other, the rod was placed through the center of the container and soldered in position. The crystal, hands and dial were then removed from the clock and the cupped end of the drum shaft was slipped over the exposed minute-hand or hour-hand shaft. Thus, the speed of the drum could be regulated for one complete revolution per hour or per twelve hours.

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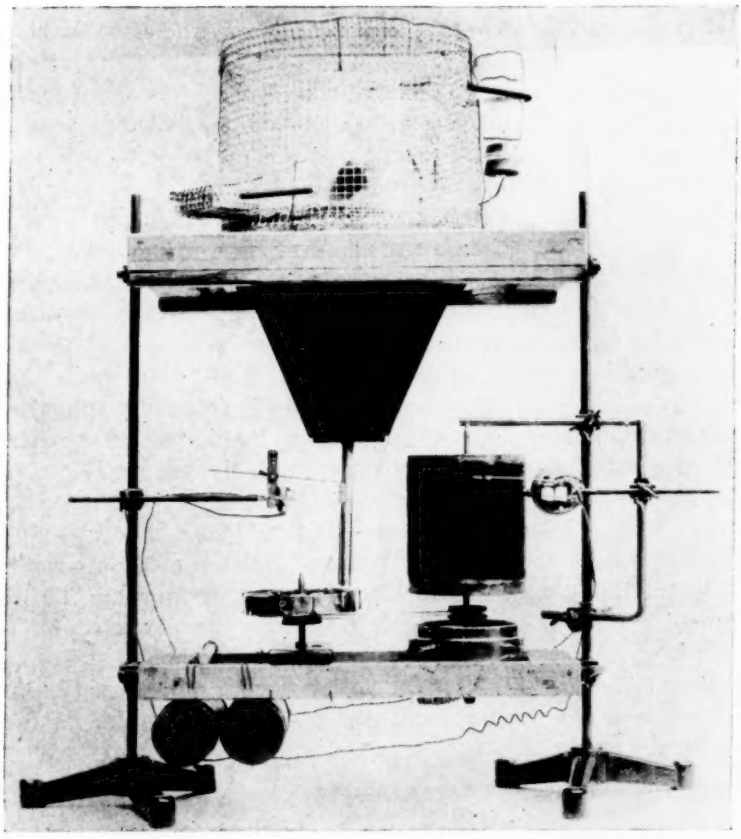

Fig. 1.—Feceometer in complete assembly and operation.

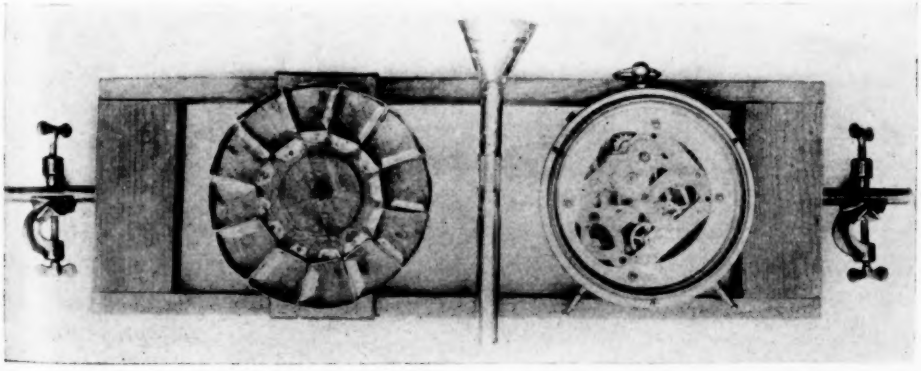

Fig. 2.—Lower open-frame shelf (downward view). Showing feces-collecting wheel with compartments (left); Big Ben clock with crystal, hands and dial removed (right); and metal feces-collecting funnel with open window in the neck (middle).

A feces-collecting wheel was constructed by mounting twelve equal tin compartments on a five-inch shafted disk of wood (Fig. 2). Transmission was obtained through the use of grooved cork pulleys of the same diameter and a belt drive attached to the shaft of the drum.

A simple trip-pan lever was made from the balance-wheel portion of a discarded clock frame, a split piece of bamboo and a small wire brush. A mercury well was mounted on the base of this instrument for electric attachment. Trip-pans were cut from discarded photographic film to the proper shape so that they could ply freely within the funnel neck. A bamboo lever having a "film" writing point was attached to the armature of a small electric buzzer and served as the electric recorder. Two dry cells were slung on wire hangers from the under side of the lower shelf and were electrically connected to the trip-pan lever and the electric recorder.

In the operation of this apparatus, the fall of the urine is broken and dispersed by and through the wire meshes of the collecting funnel, while the feces are permitted to roll directly and uncontaminated to the funnel neck. On further descent, the trip-pan is encountered, tripped, the electric circuit is closed and the electric recorder marks the fall on the revolving smoked drum. The trip-pan having been so adjusted that the feces cannot rest upon it, immediately comes back to its former position and the electric circuit is again broken. Meanwhile, the feces continue their journey through the remainder of the funnel neck and land in their respective compartment on the feces-collecting wheel. A feces-collecting tube of glass can be inserted into the funnel neck thus replacing the feces-collecting wheel. Likewise, the kymograph may be discarded and the feces-collecting wheel be mounted in direct transmission with the clock.

SUMMARY

A method for the construction of a simple feceometer is described. It is believed that this method for studying the rate of defecation and the separation of feces in the rat under different dietary conditions is superior to any known to exist.

A PROPOSED CHEMICAL TEST FOR PYROGEN IN DISTILLED WATERS FOR INTRAVENOUS INJECTIONS*

By Edgar B. Carter, Indianapolis, Ind.

IT WAS Seibert[1] and her associates, working in the Department of Pathology of the University of Chicago, who pointed out that about 50 per cent of the distilled waters, examined from different laboratories, produced fever when injected intravenously into rabbits. They identified this fever-producing substance as the product of bacterial growth in the water and called it "pyrogen." Since this time the importance of pyrogens as a cause of febrile reactions following intravenous injections prepared from distilled water, has been emphasized by Eggleston, et al.,[2] and Titus,[3] and the experiences of many hospitals since this time have seemed to indicate that if freshly distilled water, properly prepared, is used in making solutions for intravenous injections, the usual febrile reaction does not occur.

Regardless of how carefully water is distilled and redistilled, it is practically impossible to avoid the contamination with the ordinary air-borne bacteria. Many of these bacteria grow very rapidly at warm room temperatures, and since the water is usually collected and stored near the still, it is not unusual to find a temperature varying all of the way from 25° to 30° C. The subsequent sterilization of the water may kill the bacteria but it does not remove the dead bodies of the germs nor their metabolic products. The water thus contains actual protein material which, when injected intravenously in sufficient quantities, produces chills and a resulting fever with a shock depending in severity upon the amount of the bacterial products (pyrogens) contained therein.

In searching for some test or some measure of pyrogen in distilled water intended for intravenous injection many procedures have been considered. Seibert has pointed out that a bacterial count of the water will not indicate the quantity of pyrogen. Even a sterile water may contain a large quantity. Albuminoid ammonia may be demonstrated in these waters, but the quantitative estimation of the albuminoid ammonia is a laborious procedure and is not adapted to routine use. The U. S. Pharmacopeia X gives a test for oxidizable material and directs that 100 c.c. of distilled water be heated to boiling, acidulated with 10 c.c. of diluted sulphuric acid, and treated with 0.1 c.c. of tenth normal potassium permanganate. The color is not to be destroyed by boiling for ten minutes.

Since redistilled water properly prepared should not contain any oxidizable substance, and since bacterial bodies and their metabolic products are

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readily oxidized by boiling permanganate, it would seem that this test could be adapted to the measurements of pyrogen in distilled water intended for intravenous injection. Many tests made over a long period of time indicate that it is quite easy to prepare a distilled water which will not only answer this test, but which will likewise answer the test if only 0.05 c.c. of tenth normal potassium permanganate is used. It may also be shown that such water which answers the test perfectly will quickly discharge the color imparted by the potassium permanganate after the water has been stored in a warm room overnight. In order to facilitate the measurement of the potassium permanganate solution, we have proposed the use of 0.1 c.c. of twentieth normal potassium permanganate, thus offering a test which is twice as delicate as that proposed by the U.S.P. X.

The method which we propose has been tried out for a number of months and seems to indicate that distilled waters not discharging the color are free from pyrogens and, therefore, from febrile-producing organic substances. The test is as follows:

"Heat 100 c.c. of distilled water in a clean Pyrex beaker to boiling, acidulate with 10 c.c. of dilute sulphuric acid (10 per cent) and add 0.1 c.c. of twentieth normal potassium permanganate. The color of the solution should not be destroyed by continuing the boiling for ten minutes."

The method is offered with the hope that it will be tried widely in those hospital laboratories responsible for the safety of solutions intended for intravenous use.

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ACETONE AS A YARDSTICK FOR KETOSIS*

By Arthur T. Brice, Jr., B.A., Florence, S. C.

IT OCCURRED to us that a simple yardstick applicable to a ketonuria might prove a valuable tool for the accumulation of data bearing on the problems of fat metabolism. The sensitivity of Gerhart's test[1] of not less than 50 mg. per cent did not seem suitable for our purpose. The salicylic aldehyde reactions of Benedict and Behre[2, 3, 4] are extremely sensitive tests for acetone but are not sensitive to diacetic acid which is the principal end product in urine of the faulty metabolism of ketogenic substances. These tests are also unsuited to quantitative adaptations due to the fact that different brands of the reagent are reported to show marked differences of color intensity. Lange's and Rothera's tests[5] are also unsuited to quantitative adaptation due to the difficulty in handling ammonia easily with quantitative accuracy. Our attention therefore centered upon Legal's test[6] as the most suitable for our purpose. This test offers an immediate and very clear-cut differentiation between negatives and positives, and its reading is not so often obscured by the interfering orange and brown colors encountered in the nitroprusside tests with ammonia.

EXPERIMENTAL

Series of dilutions of U.S.P. acetone in water, and in composite specimens of ketone free urine were prepared and put through the test using fixed proportions of the specimens and reagents. The tubes bearing the test solutions were diluted to a fixed value with distilled water and allowed to stand overnight at room temperature. The tubes containing acetone above a certain value were green, while those below this value were light yellow in color. The experiment was repeated a number of times with identical results. It was found possible to decrease the time of color change to the indicated end points by heating the tubes in a water-bath. We became convinced that we were dealing with compounds the formation or nonformation of which depended primarily on the quantitative proportions of the reagents involved. To check this conclusion an examination of the colors produced in the test was made in the DuBoscq colorimeter immediately after setting up the reactions and using the 10 per cent dilution of acetone as a standard. The same color split in the series was observed, though the density of color throughout the series was not directly proportional to the quantity of acetone present.

Experiments were next carried out to ascertain what proportions of specimen and the various reagents involved would bring the observed color split to a point most suitable for the examination of pathologic urines. Using the proportions thus determined, the color split occurs at approximately the

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0.75 per cent dilution of acetone. This value when put through the test shows a distinct carmine color while lower concentrations of acetone are lilac. The difference is so marked that it can almost be determined whether an unknown falls above or below this value without the use of any standard.

ARTIFICIAL STANDARDS

The problem of producing a set of artificial standards for use with this test was next undertaken in cooperation with the Hynson Westcott & Dunning Company of Baltimore, Md. This company is now prepared to place on the market such a set. It consists of nine standard tubes corresponding in color value to the following volumetric equivalents of U.S.P. acetone in urine, 5 per cent, 3 per cent, 2 per cent, 1 per cent, 0.75 per cent, 0.5 per cent, 0.25 per cent, 0.125 per cent, 0 per cent; a specimen tube of the same diameter as the standard tubes, and a small metal block comparator box for making the color comparison readings. One of these sets has been in use in this laboratory for over a year and found not to have deteriorated in color value.

TECHNIC OF THE TEST

Reagents.—Sodium hydroxide, a 20 per cent aqueous solution. Sodium nitroprusside, a 10 per cent aqueous solution. This solution must be freshly prepared and cannot be over 24 hours old, although the error in using older solutions is directly proportional to the concentration of acetone. It has been customary in this laboratory to put up 1 gram quantities in powder papers, dissolving one in 10 c.c. of distilled water before running each series of tests. Glacial acetic acid, U.S.P. 99 per cent.

Apparatus.—One set of artificial color standards. One specimen tube of the same diameter. One small block comparator box. Test tubes or cylinders graduated at 25 c.c. Volumetric pipettes capable of delivering 1 and 3 c.c.

Technic.—a. Into the test tube or graduated cylinder pipette 3 c.c. of the filtered or centrifuged specimen of urine to be examined.

b. Add the following reagents in the order and quantity indicated: 1 c.c. of 20 per cent sodium hydroxide. One c.c. of 10 per cent sodium nitroprusside, freshly prepared. One c.c. of glacial acetic acid.

Note the time of adding the glacial acetic acid.

c. Dilute at once to the 25 c.c. mark with distilled water and mix by inversion several times.

d. Pour a portion of the liquid into the small specimen tube and make the comparison with the standards in the comparator box within the time interval of from four to seven minutes after the addition of the glacial acetic acid.

The best source of light for making the color comparison is a 50 or 75 watt blue "Daylight" nitrogen incandescent bulb. The tubes in the comparator block should be held close to and directly in front of it. This light has been found to be more suitable for routine use than daylight of varying intensity.

Interpretation.—The standard most nearly matching the specimen represents the color equivalent of U.S.P. acetone present as acetone and diacetic acid in the specimen. The standards represent a series of different colors, but interpolation between the standards is possible. The rule for negatives is that the color produced in the test will exactly match that of the original filtered specimen of urine. The 0 per cent standard supplied with the set is a control standard made to reproduce the color often obtained with highly concentrated pathologic specimens, which may contain as much as the equivalent of 5 milligrams of acetone per 100 c.c.

DISCUSSION

The time interval of four minutes was allowed in preparing the standards in order to permit the technician to set up a series of tests before making the readings. It is strictly necessary to adhere to this interval in making determinations by this method, as the color values all tend to be higher than the standard values upon first setting up the reaction, and fade to values lighter than the true standards after the expiration of seven minutes. A false reaction giving a color distinctly more orange or brownish than any of the standards is occasionally met with in specimens containing large quantities of amorphous urates, and also in specimens containing very large numbers of pus cells. In our experience it has been confined entirely to the range of acetone values between 0.125 and 0.5 per cent. An examination of a dozen or more of such specimens by other and more accurate methods has shown them all to contain acetone and diacetic acid, and it has been found reliable to estimate the ketone content of such off-color specimens in our method by matching the intensity of light transmitted through the specimen and standards rather than the color value.

SUMMARY

1. A brief discussion of the adaptability of various clinical tests for ketone bodies in urine to quantitative methods of procedure is given.

2. Experimental work indicating the suitability of the Legal test as a quantitative procedure is reported.

3. The technic of this test making use of a set of artificial color standards is described.

4. A discussion of factors of error inherent in the method and of false positives which may be encountered is given.

Thanks are due to Miss Jeanette Allen Behre, Ph.D., for supplying the standards and reagent for a comparative series of checks of our method by her salicylic aldehyde distillation procedure.

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STERILIZATION OF CYSTINE CULTURE MEDIUM UNDER STEAM PRESSURE*

By Frederick W. Shaw, M.Sc., M.D., Richmond, Va.

THE use of cystine in culture medium for the growing of Bacterium tularense has been brought to the attention of bacteriologists by Francis.[1] Francis' directions for the preparation of this medium are as follows: "Beef infusion agar containing 1 per cent peptone and 1 per cent agar adjusted to a reaction having a P_H of 7.6 is kept on hand in stock. When needed, there is added to the stock agar 0.1 per cent cystine and 1 per cent dextrose, and this is placed for fifteen minutes at the temperature of streaming steam in an Arnold sterilizer to melt the agar and to sterilize the cystine, after which the medium is tubed, slanted, and incubated twenty-four hours to insure sterility."

Cystine medium prepared as above leaves some of the cystine undissolved, even though an amount as small as 100 c.c. is prepared.

In a recent note the writer[2] reported a method of preparing cystine agar whereby the cystine was dissolved in the buffer solution and the cystine agar was autoclaved at 15 pounds steam pressure for fifteen minutes. This gives a clear, sterile medium with no precipitate.

Cystine agar on which Bacterium tularense grows abundantly may be prepared by a modification of the method described by Francis. The stock beef infusion agar with cystine and dextrose is not put into the Arnold for fifteen minutes, but is placed in the water-bath at 100° C. and is allowed to remain there, with occasional shaking, until the cystine is dissolved. The time required for solution to take place varies with the amount of medium in preparation. For 100 c.c. amounts, using 0.1 per cent cystine, the time required is from ten to fifteen minutes.

Due to the relative instability of cystine at high temperatures, it was thought advisable to determine the amount of decomposition of the cystine under autoclave temperature and time (120° C. for fifteen minutes).

Hoffman and Gortner[3] show that cystine is relatively stable to long boiling in 20 per cent hydrochloric acid, the only important change which is brought about by such treatment being the racemization of the l-cystine to an inactive form.

Gortner and Sinclair[4] state that boiling cystine with 1 per cent sodium carbonate for two hours causes a loss of approximately 10 per cent, four hours 16 per cent, twelve hours 35 per cent, and twenty-four hours 50 per cent of the cystine.

Increasing the strength of the sodium carbonate solution to 5 per cent does not greatly increase the rate of extent of deamination or apparently of

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cystine destruction, although appreciably larger quantities of the cystine sulphur are converted into sulphide sulphur.

To determine the destruction of the cystine under various conditions, I conducted the following experiments:

Experiment 1.—Two-tenths gm. disodium hydrogen phosphate and 100 mg. of cystine were dissolved in 100 c.c. of distilled water. One drop of 10 per cent sodium hydroxide was added to give a P_H of 7.4. This was divided into three equal parts: one part was boiled for thirty minutes in the water-bath; another part was autoclaved at 15 pounds steam pressure for fifteen minutes, while the third part was the control. Using the Folin-Marenzi[5] colorimetric method for the determination of cystine, the destruction of the cystine was approximately 2½ per cent.

Experiment 2.—One hundred mg. of cystine were dissolved in 100 c.c. of 1 per cent sodium carbonate and autoclaved at 15 pounds steam pressure for fifteen minutes. A like solution and amount was boiled in the water-bath for fifteen minutes and then autoclaved as above. A third solution of like ingredients and quantity was boiled, only, for thirty minutes. Using the colorimetric method it was not possible within the error of the colorimeter to determine any difference in the loss of cystine by the three methods. All agreed in having a loss of approximately 2½ per cent cystine.

Experiment 3.—(A) One hundred c.c. of heart infusion agar containing 1 per cent dextrose and 0.1 per cent cystine was prepared according to the method of Francis. After fifteen minutes in the Arnold, 10 c.c. were removed, placed in a tube and centrifuged to remove some of the cystine in suspension.

(B) A similar amount of heart infusion dextrose cystine agar was prepared by dissolving the cystine in the medium by use of the water-bath at 100° C., then autoclaving for fifteen minutes.

The cystine content was then determined by the Folin-Marenzi method. It was found that the heart infusion agar without the addition of the cystine gave a blue color with this method. Therefore, the results of this experiment are expressed in millimeters. For (A) set at 20, (B) read 14.5.

This experiment shows that the loss of cystine due to the heat of the autoclave more than compensated for the loss of cystine that does not go into solution by the method described by Francis.

One may deduce from the work of Gortner and Sinclair that the destruction of cystine in the autoclave at 15 pounds steam pressure for fifteen minutes would be quite small, in fact negligible, as far as bacteriologic culture medium is concerned. The experiments reported in this paper bear out the assumption. The advantages of a clear, sterile medium, without incubation to determine the sterility, are obvious.

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METHOD FOR REPORTING AND INTERPRETING THE LEUCOCYTE COUNT*

By Fred. Boerner, V.M.D., Philadelphia, Pa.

THE purpose of a leucocyte count is to determine whether there is an increase or decrease from the normal range of any of the various types of leucocytes present in the circulating blood as well as the detection of the presence of immature or abnormal types. This cannot be accomplished merely by making a total leucocyte count, due to the fact that there can be marked deviations from normal of certain types of cells with or without an increase or decrease in the total number. It is therefore necessary to make both a total and a differential count. From the two the total number of each type of cell can be calculated. Either a total or differential alone is of little value.

Ever since the white cell count has been used for diagnostic and prognostic purposes the clinicians have been receiving incomplete reports or rather reports which need further calculations to be of value. The result is that they look first at the total count then at the various percentages, then again at the total and back to the percentages in an effort to determine whether there is anything abnormal about it. By this means unless he is a good mathematician and is familiar with the normal range of various cells he seldom detects anything except marked changes.

The custom of reporting the total and percentages separately has led the profession into methods of interpreting which are confusing and often erroneous; e.g., the term "relative leucocytosis" is in common use and in itself is really meaningless. It should therefore be abandoned as it can mean a decrease, increase or no change in the actual number of the particular type of cell to which it refers. Absolute changes are the only ones of value and this term "absolute" is used in contrast to "relative." If we confine our interpretations to absolute changes only there will be no need for qualifying a leucocytosis by either term.

It is not at all surprising to ask a group of clinicians, even surgeons, who depend so much upon changes in the neutrophiles, just how many polymorphonuclears a normal individual should have and receive in reply a grand mixture of totals and percentages many of which, if properly calculated, would not fall in the normal range. The fault lies to a great extent in the method of reporting now in common use. As a step toward the correction of this confusion a method for calculating and reporting differentials is offered and in addition tables for the use of the clinician in interpreting the report.

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LEUCOCYTE COUNT

Total Number.—Determined by the methods now in common use.

Differential Count.—The number of leucocytes examined and classified in many laboratories is 100. Yet the various texts recommend from 200 to 500 and even more in research or special cases. To decide upon a number fitting for routine work is difficult. To find even 100 cells in some cases of leucopenia is difficult and time-consuming whereas in cases of leucocytosis several hundred can be found very quickly. In cases of leucocytosis the cells which occur normally in few numbers may not be seen, due to the fact that but a small amount of blood on the smear has been examined. This is especially true when but 100 cells are counted. In such cases eosinophiles for example may not appear. To overcome this the following method has been adopted at this hospital and has proved very satisfactory:

Counts under	4000	classify 50 cells
Counts from	4–10000	classify 100 cells
Counts from	10–15000	classify 200 cells
Counts from	15–20000	classify 300 cells
Counts from	20–25000	classify 400 cells
Counts over	25000	classify 500 cells

Calculation.—Determine the number of each type of leucocytes per cubic millimeter by simple mathematics. This may at first appear time consuming to the laboratory worker but in reality takes but a minute or two. Where time is of vital importance the technician can easily be trained to use a slide rule with which the figures can be obtained in less than one minute.

Reporting.—An example of the method for reporting is as follows:

Neutrophiles	4500
Basophiles	50
Eosinophiles	250
Lymphocytes	2000
Monocytes	300
Total	7100

Interpreting.—Table I gives the low and high normal findings of each type of cell and also classifies abnormals for adults and children over five. Tables II and III give figures for children under five.

TABLE I

ADULTS AND CHILDREN OVER FIVE

TYPE OF CELL	MARKED LEUCOPENIA	SLIGHT LEUCOPENIA	LOW NORMAL	HIGH NORMAL	SLIGHT LEUCOCYTOSIS	MARKED LEUCOCYTOSIS	HEMATOPOIETIC SYSTEM
Neutrophile	Under 2000	2-3000	3000	7000	7-10000	Over 10000	Bone marrow
Basophile	–	–	0	50	50- 100	Over 100	Bone marrow
Eosinophile	0	25	50	400	4- 800	Over 800	Bone marrow
Lymphocyte	Under 500	500-1000	1000	3000	3- 5000	Over 5000	Lymphatic
Monocyte	0	Under 100	100	600	600- 1000	Over 1000	Reticuloendothelial
	Total Count		4150	11050			

TABLE II

CHILDREN FROM THREE MONTHS TO THREE YEARS

TYPE OF CELL	MARKED LEUCOPENIA	SLIGHT LEUCOPENIA	LOW NORMAL	HIGH NORMAL	SLIGHT LEUCOCYTOSIS	MARKED LEUCOCYTOSIS
Neutrophile	Under 1000	1-2000	2000	7000	7-10000	Over 10000
Basophile	–	–	0	50	50- 100	Over 100
Eosinophile	–	0	25	700	700- 1000	Over 1000
Lymphocyte	Under 3000	3-4000	4000	9000	9-11000	Over 11000
Monocyte	–	0	25	700	700- 1000	Over 1000
	Total		6050	17450		

TABLE III

CHILDREN FROM THREE TO FIVE YEARS

TYPE OF CELL	MARKED LEUCOPENIA	SLIGHT LEUCOPENIA	LOW NORMAL	HIGH NORMAL	SLIGHT LEUCOCYTOSIS	MARKED LEUCOCYTOSIS
Neutrophile	Under 1000	2-3000	3000	8000	8-10000	Over 10000
Basophile	–	–	0	50	50- 100	Over 100
Eosinophile	0	25	50	700	700- 1000	Over 1000
Lymphocyte	Under 1000	1-2500	2500	6000	6- 9000	Over 9000
Monocyte	–	0	25	700	700- 1000	Over 1000
	Total		5575	14450		

For simplicity the following nomenclature is advocated:

Neutropenia = decrease in polymorphonuclears
Basopenia = decrease in basophiles
Eosinopenia = decrease in eosinophiles
Lymphopenia = decrease in lymphocytes
Monopenia = decrease in monocytes
Neutrophilia = increase in polymorphonuclears
Basophilia = increase in basophiles
Eosinophilia = increase in eosinophiles
Lymphocytosis = increase in lymphocytes
Monocytosis = increase in monocytes

Table IV gives the usual changes in the leucocyte count in disease.

DISCUSSION

The use of numbers per cubic millimeter of blood in reporting the leucocyte count is not at all new. Most textbooks advocate its use to a limited extent. However, they have it more in the background and not directly connected with the leucocyte count.

The use of the method described for reporting and interpreting leucocyte counts has distinct advantages over the method of reporting the total count and percentages of the various types separately. These are as follows:

1. It permits the clinician to detect any change either an increase or decrease of any type of cell at a glance.

2. It familiarizes him with the normal range of the various leucocytes and to detect changes from the normal even when the total count is within the normal range. Too often marked increases and decreases of certain types of cells which are of diagnostic importance are overlooked due to the fact that the total count is normal. By the method here described the total count can be disregarded entirely.

3. Repeated counts or counts of other cases are much more readily compared.

4. Published reports or tables of leucocyte counts are much more easily studied.

TABLE IV

LEUCOCYTIC CHANGES IN DISEASE

NEUTROPHILIA	NEUTROPENIA	LYMPHOCYTOSIS	LYMPHOPENIA	EOSINOPHILIA	EOSINOPENIA	MONOCYTOSIS
Severe acute infections particularly those due to cocci	Subacute and chronic infections	Convalescence from acute coccus infections	Severe acute infection particularly those due to cocci	Convalescence from acute infections particularly those due to cocci	Severe acute infections, particularly those due to cocci	Acute infectious Mononucleosis
Acute loss of blood	Common in diseases due to bacilli	Common to bacillary infections	Common in Agranulocytosis (Malignant Leucopenia)	Parasitic Diseases		Typhoid Fever
	Splenic Anemia	Syphilis Measles		Bronchial Asthma		Hodgkin's Disease
	Agranulocytosis (Malignant Leucopenia)	Lymphatic Leucemia		Eosinophilic Leucemia		Protozoan Diseases
	Convalescence from severe acute infections					

5. Descriptions of leucocytic blood changes in texts on various diseases would be much more comprehensible. At present many of these descriptions are vague due to the fact that the range of totals are given, and then the range in percentage of one or more types of cells, leaving the reader to figure it out and guess whether the low percentage goes with the low total or high.

6. The terms "relative" and "absolute" can be done away with entirely.

CONCLUSIONS

1. A method for reporting leucocyte counts is advocated which gives the number of each type of leucocyte in one cubic millimeter of blood, and its advantages discussed.

2. Normal ranges of leucocytes per cubic millimeter and the clinical significance of changes are included in table form.

THE SIGNIFICANCE OF "SHIFT TO THE LEFT" IN DIFFERENTIAL LEUCOCYTE COUNTS AND THE NUCLEAR INDEX AS A MEANS FOR INTERPRETING AND RECORDING

I. The Nuclear Index of Normal Blood and the Influence of Age

By Jules H. Gerard, M.D.,* and Fred. Boerner, V.M.D., Philadelphia, Pa.

CONSIDERABLE attention has been given lately to the histologic changes in the polymorphonuclear cells of the blood from the standpoint of their clinical significance. In 1904 Arneth called attention to the left shift of neutrophilic leucocytes as a most important part of the histologic changes in connection with clinical diagnosis and prognosis.

The term "shift to the left" or "shifting to the left" means an increase in the immature forms as distinguished from the mature forms. It can be best understood by arranging the forms of neutrophilic leucocytes in a line according to the maturity or age, placing the youngest form, the myeloblast, at the extreme left, then the myelocytes, metamyelocytes and last, at the extreme right, the mature lobulated neutrophiles. Make a perpendicular line between the immature forms and the mature lobulated neutrophiles. An increase of immature forms will raise the number on the left side of this line and decrease it on the right. When this occurs it is called a "shift to the left."

Recent interest in "shifts to the left" has prompted us to undertake this work. We fully recognize the importance of a "shift to the left" in diagnosis and especially in prognosis. However, since there is no real means at present in use of accurately interpreting and recording the presence and de-

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gree of "shift to the left" we decided to use the nuclear index as a means to that end. It was first considered necessary to determine what may be called the normal and with that point in view the following data were compiled.

Arneth was the first to classify the polymorphonuclear neutrophiles. He divided them into five classes on the basis of the number and configuration of their nuclear lobes. The first class includes those with one round or indented nucleus. This class represents the youngest form (the young metamyelocytes). The second class includes those neutrophiles with the two lobed nucleus and so on to class five which contains those cells with five lobed nuclei. Class five according to Arneth represented the oldest form of polymorphonuclear cells.

Each class was subdivided according to the shape of the nucleus or nuclear divisions. The following figures give the form and normal percentage in each class:

Class	I.	One round or indented nucleus	5 per cent
Class	II.	Two lobed nuclei	35 per cent
Class	III.	Three lobed nuclei	41 per cent
Class	IV.	Four lobed nuclei	17 per cent
Class	V.	Five or more lobes	2 per cent

On account of the subdivisions being complicated and rather impracticable they are not used. Due to the importance of Class I cells the subdivisions may well be mentioned. The first is the "M" cells with the round nucleus which corresponds to the myelocyte; the "W" cell with a slightly indented nucleus and the "T" cell with a deeply indented nucleus. The "W" and "T" cells correspond to what we now call metamyelocytes. The percentage of each occurring normally is as follows:

"M" cells	0.0 per cent
"W" cells	0.2 per cent
"T" cells	5.0 per cent

In health the percentage of the various groups remains fairly constant but in disease a marked change may occur. This change may occur with or without a change in the total count. When there is an increase in Class I (young forms) and a decrease in the "other classes" it is called a "shift to the left." When the opposite takes place it is called a "shift to the right."

In many diseases a shift to the left occurs and is most marked in severe acute infections. It is considered an unfavorable sign whereas a shift to the right is considered favorable. When a shift to the left has occurred and is then followed by a shifting to the right toward the normal percentages the prognosis is considered favorable.

Papenheim modified Arneth's classification by recommending the use of but three groups, namely, the myelocytes, the metamyelocytes, and the mature forms. This classification is more practical than Arneth's due to the fact that a shift at one end is always at the expense of the other and furthermore, changes occurring at the left (increases or decreases of immature forms) are the ones of practical importance.

Schilling has formulated a much simpler index that should become a part of every clinical and pathologic investigation. The author divides the polymorphonuclear cells into the following groups:

(a) Myelocyte, (b) young metamyelocyte (with only the slightest indentation of the nucleus), (c) older metamyelocytes or band forms (with deep indentation but no true lobulation of the nucleus) and (d) polymorphonuclears. Schilling gives the following as the normal figures for the leucocytic blood picture; neutrophilic granular cells, 67 per cent, composed of 63 per cent polymorphonuclears and 4 per cent of old metamyelocytes (band forms) with no young metamyelocytes (young forms) present.

In the Schilling method there is no need for noting the number of lobes in the nuclei of each cell. There is also no doubt that in this scheme the cells are arranged in order of their maturity.

Schilling in his book *Das Blutbild* casually mentions what he calls "the nuclear shift index" but fails to use it and prefers to use the number of immature cells in relation to a total differential count. We feel that in this method the results of changes in the polymorphonuclear cells are obscured by changes in the lymphocytes and monocytes.

TABLE I

COUNTS ON NORMAL INSTITUTIONAL CHILDREN LESS THAN ONE YEAR OF AGE

NO.	MYELOCYTES	YOUNG FORMS	BAND FORMS	MATURE POLYS.	TOTAL POLYS.	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES	NUCLEAR INDEX
1	0	0	7	13	20	76	3	0	1	1.9
2	0	0	10	15	25	72	3	0	0	1.5
3	0	0	8	20	28	69	3	0	0	2.5
4	0	0	7	13	20	79	1	0	0	1.9
5	0	0	7	15	22	77	1	0	0	2.1
6	0	0	2	7	9	90	1	0	0	3.5
7	0	0	6	19	25	70	5	0	0	3.1
8	0	0	3	11	14	83	3	0	0	3.6
9	0	0	5	25	30	69	1	0	0	5.0
10	0	0	3	9	12	82	3	3	0	3.0
Average	0	0	5.8	14.7	20.3	76.7	2.4	0.3	0.1	2.81

TABLE II

COUNTS ON NORMAL INSTITUTIONAL CHILDREN ONE TO TWO YEARS OF AGE

NO.	MYELOCYTES	YOUNG FORMS	BAND FORMS	MATURE POLYS.	TOTAL POLYS.	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES	NUCLEAR INDEX
11	0	0	6	14	20	78	2	0	0	2.3
12	0	0	7	15	22	72	3	1	2	2.1
13	0	0	5	15	20	73	6	0	1	3.0
14	0	0	5	19	24	75	6	0	1	3.8
15	0	0	2	11	13	84	3	0	0	5.5
16	0	0	4	15	19	76	5	0	0	3.7
17	0	0	7	24	31	67	1	1	0	3.4
18	0	0	5	20	25	69	5	1	0	4.0
19	0	0	12	24	36	63	1	0	0	2.0
Average	0	0	5.9	17.4	23.3	73.0	2.8	0.3	0.4	3.31

METHOD OF DETERMINING SHIFT TO THE LEFT BY THE NUCLEAR INDEX

1. Prepare blood smear and stain as for making ordinary differential count.

2. Make an ordinary differential count but in addition keep a record of the immature cells or metamyelocytes (the young forms and the band forms) while counting the polymorphonuclear granular cells. The sum of these types of immature cells are then divided into the total of mature cells as determined by subtracting the total immature count from the total polymorphonuclear count, e.g., if there are four band forms to a total count of 66 polymorphonuclear cells the mature cells would equal 66 minus 4 = 62. Then by dividing 62 by 4 we get 15.5, a normal nuclear index.

$$\frac{\text{Number of mature cells}}{\text{Number of immature cells}} = \text{nuclear index.}$$

The normal nuclear index is 15 or over.
An index between 10 and 15 is a slight shift to the left.
An index between 5 and 10 is a moderate shift to the left.
An index below 5 is a marked shift to the left.

TABLE III

COUNTS ON NORMAL INSTITUTIONAL CHILDREN TWO TO THREE YEARS OF AGE

NO.	MYELOCYTES	YOUNG FORMS	BAND FORMS	MATURE POLYS.	TOTAL POLYS.	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES	NUCLEAR INDEX
20	0	0	6	26	32	62	4	2	0	4.3
21	0	0	2	13	15	83	2	0	0	6.5
22	0	0	11	32	43	56	1	0	0	2.9
23	0	0	6	25	31	66	3	0	0	4.1
24	0	0	5	20	25	68	7	0	0	4.0
25	0	0	6	26	32	66	2	0	0	4.3
26	0	0	6	21	27	69	3	1	0	3.5
27	0	0	7	15	22	71	3	2	2	2.1
28	0	0	9	30	39	55	3	2	1	3.3
29	0	0	5	15	20	74	6	0	0	3.0
30	0	0	10	32	42	52	4	2	0	3.2
Average	0	0	6.6	23.2	29.0	65.7	3.4	0.8	0.2	3.74

TABLE IV

COUNTS ON NORMAL INSTITUTIONAL CHILDREN THREE TO FOUR YEARS OF AGE

NO.	MYELOCYTES	YOUNG FORMS	BAND FORMS	MATURE POLYS.	TOTAL POLYS.	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES	NUCLEAR INDEX
31	0	0	7	27	34	58	5	1	2	3.9
32	0	0	10	54	64	51	4	1	0	5.4
33	0	0	8	37	45	52	2	1	0	4.6
34	0	0	5	18	23	75	2	0	0	3.6
35	0	0	5	24	29	69	2	0	0	4.8
36	0	0	4	24	28	62	5	4	1	6.0
37	0	0	7	28	35	62	2	2	0	4.0
38	0	0	5	21	26	72	1	1	0	4.2
39	0	0	7	25	32	64	2	2	0	3.5
40	0	0	5	24	29	67	2	2	0	4.8
Average	0	0	6.3	28.2	34.5	61.2	2.7	1.4	0.3	4.48

This method is recommended for routine work and the results can be reported either as a shift to the left or by the resulting nuclear index. In publishing results it was thought advisable to report the findings in more detail. This has been done in Tables I, II, III, IV, and V.

Discussion.—Tables I, II, III, and IV show that in early childhood there is normally a marked "shift to the left." This varies from an average of

TABLE V

COUNTS ON NORMAL ADULTS

NO.	MYELOCYTES	YOUNG FORMS	BAND FORMS	MATURE POLYS.	TOTAL POLYS.	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES	NUCLEAR INDEX
1	0	0	4	61	65	26	2	6	1	15.0
2	0	0	2	65	67	29	2	2	0	32.5
3	0	0	3	65	68	27	5	0	0	21.3
4	0	0	4	68	72	26	0	2	0	17.0
5	0	0	3	63	66	33	1	0	1	21.0
6	0	0	1	70	71	23	2	3	0	70.0
7	0	0	3	62	65	33	1	1	0	20.6
8	0	0	4	74	78	19	1	1	1	18.5
9	0	0	3	60	63	32	3	1	1	20.5
10	0	0	3	55	58	36	5	1	0	18.3
11	0	0	2	64	66	33	1	0	0	32.0
12	0	0	2	60	62	37	1	0	0	30.0
13	0	0	2	56	58	32	6	2	2	28.0
14	0	0	4	61	65	31	3	1	0	15.2
15	0	0	2	56	58	38	2	2	0	28.0
16	0	0	4	62	66	31	3	0	0	15.5
17	0	0	2	48	50	45	0	2	3	24.0
18	0	0	4	68	72	25	1	1	1	17.0
19	0	0	4	63	67	31	2	0	0	15.7
20	0	0	3	67	70	27	0	2	1	22.3
21	0	0	1	61	62	33	3	1	1	61.0
22	0	0	2	50	52	45	3	0	0	25.0
23	0	0	1	59	60	36	1	1	2	59.0
24	0	0	3	73	76	24	0	0	0	24.3
25	0	0	2	59	61	39	0	0	0	29.5
26	0	0	0	62	62	34	2	1	1	62.0
27	0	0	3	61	64	32	2	2	0	20.3
28	0	0	1	53	54	36	2	8	0	53.0
29	0	0	2	59	61	34	5	0	0	29.5
30	0	0	2	65	67	32	1	0	0	32.5
31	0	0	0	57	57	32	3	8	0	57.0
32	0	0	0	58	58	40	2	0	0	58.0
33	0	0	2	66	68	27	3	0	2	33.0
34	0	0	0	57	57	40	3	0	0	57.0
35	0	0	1	56	57	36	1	5	1	56.0
36	0	0	1	52	53	45	2	0	0	52.0
37	0	0	2	60	62	36	1	1	0	30.0
38	0	0	2	64	66	33	1	0	0	32.0
39	0	0	2	56	58	38	3	1	0	28.0
40	0	0	1	51	52	41	5	2	0	51.0
41	0	0	0	55	55	40	1	4	0	55.0
42	0	0	2	55	57	41	2	0	0	27.5
43	0	0	1	48	49	46	1	4	0	48.0
44	0	0	1	55	56	42	2	0	0	55.0
45	0	0	2	60	62	37	1	3	0	30.6
46	0	0	2	41	43	54	3	0	0	20.5
47	0	0	1	41	42	54	1	3	0	41.0
48	0	0	2	65	67	30	2	1	0	32.5
49	0	0	2	58	60	37	2	1	0	29.5
50	0	0	4	77	81	17	2	0	0	19.2
Average	0	0	2.0	59.6	61.7	34.5	2.0	1.4	0.36	33.6

2.81 in children less than one year of age to 4.48 in children three to four years of age. At what age the nuclear index returns to the normal of 15 or over we are unable to state. This is a subject for a later paper.

Table V is for adults and is computed according to the nuclear index. Fifty adults, mostly laboratory workers, were taken as normals. Only those who were free from disease at the time and who had had no disease for one year were taken.

It will be seen from Table V that the normal nuclear index varies between 15 and 70 with an average of 33.6. We therefore consider a nuclear index of 15 or over as a normal figure.

Advantages of Nuclear Index Method Over Other Methods.—

1. The nuclear index method of determining a shift to the left is a simple procedure requiring scarcely more time than an ordinary differential count.

2. It is simple to report.

3. It is possible to follow slight changes at a glance.

4. It is not influenced by cells from hematopoietic organs other than the bone marrow.

5. It constitutes a definite criterion by which to gauge the degree of shift to the left.

6. The nuclear index indicates a shift quantitatively rather than qualitatively. In other words it indicates the increases and decreases in young forms but does not give the quality of the young forms in their degree of maturity. It seems to us that for clinical purposes this is sufficient. In cases where more detailed information is necessary the various types of young forms can be enumerated separately.

CONCLUSIONS

1. The nuclear index is a simple means of determining the shift to the left.

2. It is definite in determining the degree of shift to the left.

3. The normal nuclear index varies according to age. In adults it is 15 or over. In children there is a marked shift to the left up to three or four years of age.

4. Due to its simplicity the shift to the left should be done routinely at the time of the differential count.

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THE SIGNIFICANCE OF "SHIFT TO THE LEFT" IN DIFFERENTIAL LEUCOCYTE COUNTS AND THE NUCLEAR INDEX AS A MEANS FOR INTERPRETING AND RECORDING

II. The Nuclear Index in Disease

By Jules H. Gerard, M.D.,* and Fred. Boerner, V.M.D.,
Philadelphia, Pa.

IN A PREVIOUS paper we have advocated the use of the nuclear index as a method of interpreting and recording the "shift to the left" of the neutrophiles. The normal findings (indices) are given for children up to four years of age and for adults. The nuclear index of 15 or over was found in normal adults and an average of from 2.81 for children under one year of age to 4.48 for children under four years of age. For the purpose of further demonstrating the value of this method in reporting shifts and in addition furnishing further information regarding shifts in various diseases we determined the shift in a variety of diseases and used the nuclear index as a means of determining the degree. The cases were taken at random and differential counts made with special attention to the shifts and without regard to the total counts. In some cases repeated counts were made and the progress of the shift noted.

As stated in a previous paper the nuclear index is determined by dividing the number of immature neutrophiles into the number of mature neutrophiles. In other words it is a proportion of mature to immature cells as determined by the differential count.

In this paper we will give the nuclear index of various diseases showing, in general, how an acute infection lowers the nuclear index.

Table I indicates the nuclear index in one disease only, namely, lobar pneumonia.

It will be seen from Table I that in Case 1 there was a nuclear index of 1.5 which gradually rose to 4.9, which is normal for a child of three to four years of age and indicates recovery. The child recovered. There was just one nuclear index computed in Cases 2 and 6 which was 3.5 and 1.8 respectively but indicated what might be expected in a case of lobar pneumonia in an adult where a nuclear index showing a marked "shift to the left" is the usual finding.

In Case 3 which was also in an adult there was a marked "shift to the left" with a nuclear index of 3.8 which fell to 2.4 and then rose to 4.2 and

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then fell again to 1.9 following the onset of empyema. In Case 4 there was a gradually rising nuclear index from 1.8 to 5.4 which was indicative of recovery. The patient recovered. In Case 5 the nuclear index fell from 2.7 to 0.93 and then rose again to 3.7. This would indicate recovery which actually took place in this case. Case 7 shows a nuclear index of 0.83 which is a marked "shift to the left" for a child of four years of age. This patient had a frank case of lobar pneumonia.

It will be noted that the eosinophiles were markedly diminished or absent altogether in the above cases of lobar pneumonia except in one count made on Case 4 where they were 3 per cent. Eosinophiles are known to be greatly diminished or absent altogether in cases of severe acute infections.

TABLE I*

LOBAR PNEUMONIA

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	REMARKS
1	17300	0	6	26	58	90	10	0	0	0	1.5	Child
1	12500	0	0	8	25	33	64	2	0	1	3.1	Child
1	9400	0	0	12	47	59	38	2	0	0	3.9	Child
1	8200	0	0	6	29	35	63	2	1	0	4.9	Child
2	23800	0	4	16	71	91	7	1	1	0	3.5	Adult
3	9000	1	3	14	69	87	11	2	0	0	3.8	2/24/30
3	9000	1	5	18	59	83	13	4	0	0	2.4	2/25/30
3	46000	0	1	15	77	93	5	2	0	0	4.2	3/ 3/30
3	31360	0	1	27	55	83	14	3	0	0	1.9	3/17/30 Empyema
4	25800	0	3	29	57	89	11	0	0	0	1.8	
4	18300	0	1	28	57	86	11	2	0	1	2.0	
4	9000	0	1	9	54	64	15	18	3	0	5.4	
5	17100	0	3	21	67	91	7	2	0	0	2.7	
5	22300	0	2	45	44	91	8	1	0	0	0.93	
5	21600	0	1	18	71	90	6	4	0	0	3.7	
6	20900	0	6	25	57	88	9	2	0	1	1.8	
7	23550	0	5	37	35	77	21	1	0	1	0.83	4 years of age

*To facilitate the making of the numerous tables the following symbols will be used: M, for myelocyte, Y, for young forms, B, for band forms, M. P., for mature polys., T. P., for total polys., L, for lymphocytes, Mo, for monocytes, E, for eosinophiles, Ba, for basophiles, N. I., for nuclear index.

TABLE II

PULMONARY TUBERCULOSIS

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	REMARKS
8	—	0	0	37	33	70	26	4	0	0	9.4	
9	11600	0	0	10	75	85	11	3	0	1	7.5	
10	13050	0	0	26	51	77	14	6	1	2	1.9	
11	7700	0	0	24	37	61	36	1	0	2	1.5	
12	7600	0	1	30	48	79	21	0	0	0	1.5	

It will be noted in Table II that Cases 8 and 9 showing a nuclear index of 9.4 and 7.5 respectively indicate a moderate "shift to the left" whereas Cases 10, 11, and 12 show a nuclear index of 1.9 to 1.5 which indicates a marked "shift to the left." These cases were not followed on the wards.

TABLE III

TUBERCULOSIS OTHER THAN PULMONARY

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	REMARKS
13	14000	0	0	25	56	81	10	8	0	1	2.2	
14	11000	0	0	23	45	68	30	1	0	1	1.9	

In Table III Case 13 showing a nuclear index of 2.2 or a marked "shift to the left" was a case of genitourinary tuberculosis. Case 14 also showing a marked "shift to the left" (nuclear index 1.9) was a case of tuberculous meningitis.

TABLE IV

MASTOIDITIS

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	REMARKS
15	12400	0	0	9	71	80	16	3	1	0	7.9	
16	8100	0	0	11	57	68	30	0	0	2	5.2	Age 12 yr.
17	47300	0	2	41	37	80	17	3	0	0	0.86	Age 8 mo.
18	27700	0	0	43	42	85	15	0	0	0	0.9	Age 3 yr.
18	28900	0	2	29	50	81	16	2	1	0	1.6	

Cases 15 and 16 in Table IV show a nuclear index of 7.9 and 5.2 or a moderate "shift to the left" while Case 17 shows a marked "shift to the left" (nuclear index 0.86). Case 18 shows a rising nuclear index of from 0.9 to 1.6 indicating recovery. The patient recovered.

TABLE V

APPENDICITIS

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	REMARKS
19	16400	0	0	9	77	86	12	2	0	0	8.5	
20	20200	0	1	18	73	92	8	0	0	0	3.8	
21	17400	0	0	12	56	78	15	4	3	0	4.7	

In Table V, Case 19 with a nuclear index of 8.5 shows a moderate "shift to the left" whereas Cases 20 and 21 with a nuclear index of 3.8 and 4.7 respectively show a marked "shift to the left."

The above cases are self-explanatory; those with a nuclear index of 1 to 5 show a marked "shift to the left"; those between 5 and 10 show moderate "shift to the left"; those between 10 and 15 show a slight "shift to the left" and those over 15 no "shift to the left."

Case 22 is one of acute sinusitis as distinguished from Cases 23 and 24 which are chronic sinusitis. Likewise Case 30, acute salpingitis, is distinguished from Case 31, chronic salpingitis. It would seem from these two examples listed above that acute and chronic infections have different responses as manifested by the nuclear index. However, the cases are much

TABLE VI

MISCELLANEOUS

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	DISEASE
22	13100	0	3	19	53	75	21	4	0	0	2.4	Acute sinusitis
22	12900	0	0	16	52	68	27	4	1	0	3.2	Acute sinusitis
23	10200	0	0	6	80	86	12	2	0	0	13.3	Chronic sinusitis
24	7800	0	0	4	48	52	42	4	1	1	12.0	Chronic sinusitis
25	—	0	0	6	73	79	19	0	1	1	12.5	Cold
26	12400	0	5	40	47	92	8	0	0	0	1.0	Pneumonitis
27	15000	0	0	14	71	85	13	2	0	0	5.0	Infected hand
28	6300	0	0	5	54	59	36	3	0	2	10.8	Chronic ulcerations
29	10000	0	1	6	62	69	29	2	0	0	8.8	Multiple arthritis
30	8500	0	0	10	48	58	33	7	2	0	4.8	Acute salpingitis
31	5850	0	0	3	56	59	39	0	0	2	18.6	Chronic salpingitis
32	—	0	0	14	57	71	22	6	0	1	4.0	Suppuration of hip
33	14300	0	1	14	66	81	16	3	0	0	4.4	Post operative hemorrhage
34	6600	0	0	4	50	54	41	3	2	0	12.5	Arthritis
35	2600	0	0	10	52	62	34	4	0	0	5.2	Generalized lymphadenitis
36	9700	0	1	22	49	72	27	1	0	0	2.1	Streptococcus infection
36	6400	0	0	20	61	81	19	0	0	0	3.0	Streptococcus infection
37	20700	0	3	27	56	86	12	2	0	0	1.8	Erysipelas
38	15600	0	10	29	14	53	42	5	0	0	0.31	Colitis—age 9 yr.
39	11200	0	1	15	33	49	50	1	0	0	2.0	Bronchial pneumonia. Age 9 mo.
40	10200	0	0	15	57	72	25	2	1	0	3.8	Otitis media
41	7600	0	2	37	52	91	4	5	0	0	1.3	Meningitis
42	4000	0	0	6	55	61	38	0	1	0	9.1	Hyperthyroidism
43	19600	0	0	27	56	83	14	2	1	0	2.0	Pyelitis
44	14000	0	0	17	67	84	14	2	1	0	3.9	Acute rheumatic fever

too few to make any definite statement. Case 31 fails to show a nuclear index of 1 to 15 and hence must be called a normal nuclear index.

By comparing the nuclear index with the total white count it will be seen that the nuclear index may indicate a "shift to the left" without an increase in the total white count.

TABLE VII

CASE NO.	TOTAL WHITE COUNT	M	Y	B	M. P.	T. P.	L	MO	E	BA	N. I.	DISEASE
45	7000	0	0	2	56	58	35	1	6	0	28.0	Hodgkin's disease
46	9100	0	0	3	56	59	38	1	1	1	18.6	Hodgkin's disease
47	9100	0	0	3	70	73	25	1	1	0	23.3	Sacro-iliac strain
48	7100	0	0	2	63	65	35	0	0	0	31.5	Cholelithiasis
49	9400	0	0	10	39	49	49	2	0	0	3.9	Pul. collapse—age 2 yr.

Table VII shows what might be expected in cases where there is no acute infection. Cases 45 and 46 show what may be expected in cases of Hodgkin's disease showing a nuclear index of 28.0 and 18.6; a normal nuclear index and hence no "shift to the left." Cases 47 and 48 show likewise a normal nuclear index while Case 49 with a nuclear index of 3.9 is in a child two years of age and hence cannot be considered a "shift to the left."

CONCLUSIONS

1. The nuclear index may indicate a "shift to the left" without an increase in the total white count.

2. A nuclear index below 15 indicates an infection.

3. The nuclear index is recommended for interpreting and recording "shifts to the left" of the neutrophilic leucocytes.

4. The findings are readily comparable and changes in the "shift" are easily detected.

5. Indices made in various laboratories would have precisely the same significance and would help to standardize the method of reporting "shifts."

A TECHNIC TO FACILITATE READING OF KAHN RESULTS IN HEMOLIZED, HIGHLY TINTED OR CLOUDY SERA, AND IN SERA OF DOUBTFUL REACTION*

By B. S. Levine, Ph.D., Hines, Illinois

WE HAVE frequently encountered difficulty in reading the results of the Kahn precipitation reaction in sera highly tinted by hemolysis, particularly with specimens of blood forwarded from distant points; with sera of a turbid or chylous character, or doubtful reactions. Several methods have been suggested to rectify these conditions. Leiboff[1] found that the difficulty in reading weakly positive reactions is not entirely due to the number and size of the particles, but to the extraneous substances which obscure them, and suggested centrifugalizing the tubes, pouring off the supernatant fluids and resuspending the sediment in clear saline. Levine[2] centrifugalized tubes showing doubtful results and tested the precipitates for their ability to fix complement using the cold incubation procedure of Kolmer.[3] In another paper the author reported the use of a procedure for experimental purposes based upon the complete precipitation of the entire antigen by treating the completed Kahn test with distilled water, centrifugalizing the precipitate and after separating the supernatant fluid, testing it for ability to fix complement by warm incubation.[4]

We tried the method suggested by Leiboff, and found that the extraneous fine matter was carried over into the clear saline, and the difficulty of deciding whether or not the suspended matter was the result of the immunologic reaction was not obviated. The two procedures proposed by Levine appear to be more logical from the theoretical viewpoint. Objection may be raised to this procedure as constituting a decided deviation from the simple Kahn test.

It became desirable, therefore, to devise a method which would remain true to the principles of the original test in every particular, yet one which

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would eliminate all doubt in the final decision. The following has been worked out in the Edward Hines, Jr. Hospital laboratory and has given satisfactory results in tinted as well as in turbid and doubtfully reacting sera.

A volume of the serum (1 c.c.) is placed in a tall serologic tube, and a wax pencil mark is made to indicate the meniscus of the fluid. Two parts of saline (in this instance 2 c.c.) are now added to the tube, and the contents well mixed. The tube now contains three volumes (3 c.c.) of fluid. To this are added two parts (2 c.c. in this case) of a saturated solution of ammonium sulphate, and the contents of the tube shaken. The tube is now centrifuged at *high* speed (about 3000 r.p.m.) for fifteen minutes. The precipitate must become *well* packed at the bottom of the tube. The supernatant fluid is then thoroughly drained and saline added to the wax pencil mark, thus bringing the contents of the tube to the original volume of the serum (1 c.c.). The tube is shaken until the entire precipitate is dissolved. It is again well centrifugalized to remove all extraneous suspended matter, the clear fluid poured into another tube, and used in the precipitation test which is conducted in exactly the same manner as prescribed by Kahn for serum.[5] That is, into each of three Kahn tubes antigen-saline mixture is placed in 0.05 c.c., 0.025 c.c., and 0.0125 c.c. quantities; then to each of the tubes are added 0.15 c.c. of the clear and colorless fluid containing the dissolved precipitate; the tubes are shaken for three minutes; 1 c.c., 0.5 and 0.5 c.c., saline added respectively; the tubes again shaken for a short time, and the results read.

Highly tinted sera are rendered practically colorless, and chylous and turbid sera become clear.

Bile stained sera are cleared of confusing substances and of the stain.

The procedure is in accord with the Kahn test in every respect.

Doubtful reactions occasionally change in their intensity to become stronger or slightly weaker, but are clear cut wherever the precipitate is the result of interplay of immunologic factors.

SUMMARY

A technic to facilitate reading of Kahn results in hemolyzed and highly tinted sera and in sera of doubtful reaction is described. It is not a routine test and is not intended to clarify cases of doubtful clinical diagnosis.

Acknowledgment is made to Dr. L. H. Prince, Pathologist at this station.

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AN APPARATUS TO FACILITATE THE PREPARATION OF FRESH FROZEN SECTIONS*

By Harold D. Caylor, M.D., and Truman E. Caylor, M.D., Bluffton, Ind.

MOST hospitals, and especially smaller hospitals, have inadequate arrangements and space for making rapid frozen sections for the diagnosis of tissue removed during operations. We have devised an inexpensive apparatus which fills this need in our practice.

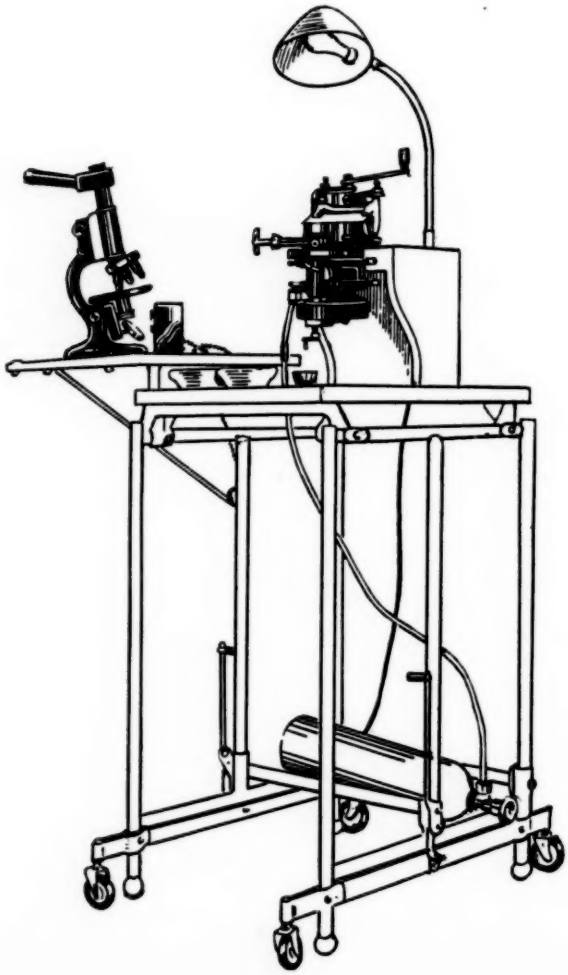

Fig. 1.

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The essential items are a freezing microtome, a microscope with a demonstrating double ocular, an adding machine stand, a small CO_2 tank, electrical connections for two lamps, one microscope lamp and one light for illumination of the microtome.

The adding machine stand is used because if the castors are in contact with the floor the apparatus can be moved about easily; on the other hand, if sections are to be made, a lever can be thrown which brings four adjustable legs in contact with the floor and renders the apparatus immovable.

A wooden platform 20½ by 17 inches is mounted on top of this stand. The common wooden mounting bracket for a freezing microtome is screwed to this top. (Fig. 1.)

A light with a flexible shaft is fastened onto the back of the microtome bracket for illumination during the section cutting procedure. On the back of the platform behind the microtome base there is a double socket which furnishes the electrical connections for the microscope light and for the light with the flexible shaft. To the front of the platform ahead of the microtome there is ample space for staining dishes and work space. A "D" tank of carbon dioxide fits across the base of the adding machine stand and is connected with the microtome by a piece of metal tubing. (Fig. 1.)

There is a drop leaf shelf, a part of the adding machine stand, on which the microscope and lamp are placed. The demonstrating double ocular replaces the ordinary ocular of the microscope.

This simple apparatus has these particular advantages: (1) It is portable, yet when the legs are let down it is rigid enough for satisfactory work. (2) It is compact; with the microscope and microtome removed and the drop leaf shelf let down, the outfit stores in a very small space. (3) Within a few minutes after the tissue has been removed, it can be sectioned and stained by a technician and examined by a pathologist. (4) The surgeon or his assistants without breaks in their surgical asepsis may observe the sections through the demonstrating ocular.

Usually from twelve to fifteen pieces of tissue can be frozen and cut before a full tank becomes exhausted.

303 South Main Street.

A USEFUL COVERSLIP HOLDER*

By S. A. Petroff, Ph.D., Trudeau, N. Y.

THE coverslip method of blood preparation is unquestionably the most efficient for differential count. The fragility and size of the thin glass makes it rather difficult to manipulate, and probably for this reason it has not been extensively used.

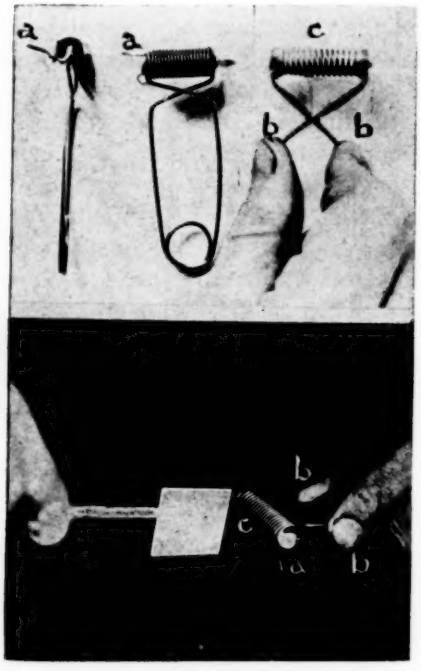

Fig. 1.

Having in mind the above objections, we attempted to design a coverslip holder which can be made with no difficulty. A brass coil spring is made by winding 18-gauge spring wire around a 7 mm. round rod. The coil is cut so that each holder will consist of approximately 10 or 12 loops. This coil spring is soldered between the ends of a cross wire spring (see Fig. 1). On the one end of the coil spring, another small piece of wire (*a*) is soldered leading from one end of the coil bent at right angles and running across, in a position to act as a guide in preventing the coverslips from going too far in.

After the blood smear has been prepared on the coverslips, they are marked and dried in the air. By pressing the opposite sides (*b*), the loops

*From the Research and Clinical Laboratory, Trudeau Sanatorium.
Received for publication, May 31, 1930.

in the coil (*c*) are separated, and in each interspace the coverslips inserted. Releasing the main outside wire (*b*) the spring loops will return to their normal position, clamping the coverslips firmly. The loaded holder with the coverslips is stained by placing first in a jar containing Wright's stain for one minute, then transferred in the differential stain, which consists of equal parts of Wright's stain and distilled water for two minutes, after which they are rinsed for a few seconds in a third jar containing distilled water. The excess water from the stained coverslips can be removed, to a great extent, by touching the lower end of the coverslips on a blotting paper. The coverslips may be removed from the holder by pressing the two ends (*b*), releasing the pressure, and with forceps, placed on the blotting paper, blotted, mounted, and examined.

The advantages of this method are:

1. A large number of preparations can be stained at the same time.
2. They are stained uniformly.
3. The breakage is reduced to the minimum.

The two stains will keep well for at least two weeks.

A NEW CULTURE FLASK*

By S. A. Petroff, Ph.D., Trudeau, N. Y.

THE difficult problem of eliminating contaminations during the manipulation of Petri dish cultures, and especially where they were under observation for a long period of time was, in part, solved by the method which the author described in a previous paper.† However, regardless of the care taken, contaminations did occur in tubercle bacillus cultures. The incorporation of gentian-violet with the medium eliminated considerably the gram-positive organisms, but until recently we were not able to eliminate contamination with molds.

Recently, we have adopted a flat flask with approximately the same surface media area as the Petri dish, stoppered with cotton plugs and sealed either with wax or rubber caps. The flask is a modification of Kolle's flask of 100 mm. in diameter and 15 mm. deep. In designing this flask we have taken into consideration some points which are advantageous: the end has no corners and is round, which facilitates cleaning, washing and eliminates breakage. The lower part of the mouth (near the main part) is slightly constricted, preventing the cotton plug from being pushed into the flask.

In filling the flask with medium, a specially designed funnel is used (see Fig. 1), the outlet of which is bent at right angles, so that it can be inserted in it. The flask must be in a horizontal position, the mouth slipped over the outlet of the funnel and 35 c.c. of medium introduced, then coagulated in an

*From the Research and Clinical Laboratory, Trudeau Sanatorium.

†Petroff, S. A.: Microbic Dissociation: The Tubercle Bacillus, Proc. Soc. Exper. Biol. & Med. 24: 632-634, 1927.

Received for publication, May 31, 1930.

inspissator. The flasks are sealed by slipping rubber nipples over the mouth to prevent dehydration. The small holes in the nipples will supply sufficient oxygen for ordinary purposes.

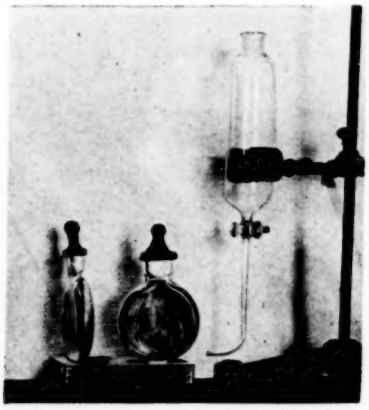

Fig. 1.

The cleaning of the flask is a very easy matter. After the old cultures have been sterilized in the autoclave, the medium can be removed from it with a rod approximately $\frac{3}{16}$ inches in diameter and 10 inches long, one end bent at 120°, to which a rubber policeman is attached.

APPLICATION OF THE QUINHYDRONE METHOD FOR THE DETERMINATION OF THE P_H OF SOLID MEDIUM*

By William Steenken, Jr., Trudeau, N. Y.

THE recent studies on dissociation of the tubercle bacillus by Petroff and associates[1] revealed that the P_H of the fluid medium was one of the important factors for the development of a certain type of colonies. Therefore, it seems logical that the P_H of the solid medium (gentian-violet-egg, which is extensively used in the growing of the tubercle bacillus) should be titrated and adjusted to the proper P_H in order to obtain a constant result.

The method which is about to be described is a modification of Paul D. Watson's[2] method used in determining the P_H of cheese.

METHOD

The P_H of the medium should be adjusted 0.3 to 0.4 lower than the desired P_H by electrometric titration before inspissation. After inspissation, when the medium is completely cooled, cut small portions (5 × 5 mm.) from different sections of one of the plates, place in a porcelain mortar that has

*From the Research and Clinical Laboratory, Trudeau Sanatorium.
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been cleaned with cleaning solution and thoroughly washed with distilled water until neutral to phenol red. Add 0.2 gm. of powdered quinhydrone and triturate with the medium thoroughly to a pasty consistency.

Cullen and Biilmann's[3] electrode is used to determine the P_H. The aperture at the end of the sleeve is made slightly larger than the originators', and the bulb attached to the side arm of the sleeve is removed to prevent back pressure. The mixture of medium and quinhydrone is then worked with spatula into the aperture at the end of the sleeve that covers the electrode. The electrode is then inserted into the sleeve from the upper end until the platinum point is completely embedded in the medium. This is then placed into a small tube (10 × 30 mm.) containing a saturated KCl solution which acts as a bridge, and the E. M. F. determined.

Formula used (Cullen and Biilmann) for calculating P_H is:

$$P_H = \frac{0.4529 - \text{E. M. F.}}{0.00019837\ T.}$$

RESULTS OF DETERMINATIONS

Example—

P_H OF PETROFF'S GENTIAN-VIOLET MEDIUM BEFORE AND AFTER INSPISSATION

	BEFORE	AFTER	DIFFERENCE
P_H	6.84	7.15	+0.31
P_H	6.85	7.18	+0.33
P_H	6.87	7.18	+0.31

The question whether Donnan's equilibrium (Loeb[4]) played an important part in determining the true P_H of the medium was studied. Small portions of the medium were removed from various sections of the plate and the P_H determined. From the same plate some other portions were removed and squeezed to separate the liquid as much as possible, and the P_H was determined on the fluid and the solid. The difference of the two was plus or minus 0.05 P_H, suggesting a Donnan effect, but not enough to make the method impractical.

SUMMARY

The quinhydrone method may be used for determining P_H on solid medium, such as agar, Petroff's medium, egg medium or any other solid medium when there are no secondary oxidation or reduction reactions. If the secondary oxidation or reduction reactions are sufficiently slow the E. M. F. may be determined before such takes place.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEPROSY: Method of Distinguishing Between Living and Dead Bacilli, Aoki, T., and Aoki, Y. Japanese J. Dermat. & Urol. 29: 64, 1929.

The authors describe a method of staining which they think will distinguish between living and dead lepra bacilli.

The specimen is first stained with erythrosin 1.0, picric acid 0.15 and distilled water 100 parts for five minutes at 80°. After washing it is placed for one minute in caustic potash 5.0, absolute alcohol 30 and distilled water 70 parts, washed again and then stained for one minute in 1 per cent caustic potash 1 part, saturated alcoholic solution of methylblue 15 parts and distilled water 100 parts. The living bacilli are said to be stained a bright red, dead ones a dark violet blue and intermediate ones a light reddish violet to a blue violet color.

OLIOGENDROGLIA: Staining of, Penfield, W. Am. J. Path. 6: 445, 1930.

FIXATION

Fresh tissue should be fixed in the following solution for two hours or up to one or two days.

Fixative (F.U.P.I.)	Formalin (40 per cent commercial)	20 c.c.
	Urea	4 gm.
	Potassium iodide	6 gm.
	Water (doubly distilled)	80 c.c.

Cut sections at about 15 microns on the freezing microtome and place in distilled water.

STAINING METHOD

1. Wash in two dishes of distilled water, the first containing 10 drops of ammonia.
2. Stain in undiluted silver carbonate from one minute to one and one-half hours.

The solution is del Rio-Hortega's undiluted ammoniacal silver carbonate made up carefully as follows:

Solution of silver nitrate (Merck) 10 per cent	5 c.c.
Solution of sodium carbonate (pure) 5 per cent	20 c.c.
Ammonium hydroxide (sufficient to dissolve precipitate)	

The ammonium hydroxide, as indicated above, should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. Finally, filter and place in a dark bottle, where it will keep for long periods.

3. Wash rapidly in 60 per cent alcohol. The section should be carried through with a small anulated glass rod so as to allow all of it to be washed equally, without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.
4. Reduce by passing sections directly into 1 per cent formalin.
5. Wash in distilled water.
6. Tone by placing sections in gold chloride toning bath (Gold chloride [yellow] 1 gm.; distilled water 500 c.c.) ten or fifteen minutes until they become purple-gray in color.
7. Fix in 5 per cent hyposulphite of soda for one-half minute or more until sections are flexible.
8. Wash in water.
9. Dehydrate in dishes of graded alcohol followed by clearing in carbol-xylol-creosote (carbolic acid 10 c.c., creosote 10 c.c., xylol 80 c.c.).
10. Mount on slide in Canada balsam.

OVA: Preservation of, Sankin, S. L.: Arch. f. Schiffs- u. Trop-Hyg. 33: 416, 1929.

Rub feces up in 10 per cent acetic acid; spread in a thick film; dry in the air; add cedar oil and a cover.

TISSUE: A Gelatin Fixative for Paraffin Sections, Haupt, A. W. Stain Tech. 5: 97, 1930.

The gelatin fixative is prepared as follows: Dissolve 1 gm. of gelatin in 100 c.c. of distilled water at 30° C. Then add 2 gm. of phenol crystals and 15 c.c. of pure glycerin. Stir well and filter.

Only the best grade of gelatin should be used, and the temperature should not be allowed to exceed 30°. Gelatin which dissolves at a higher temperature is not satisfactory. If a day or two is required for the gelatin to dissolve, decomposition will probably begin, and if the phenol is added too soon the undissolved gelatin will turn white and harden.

TAPEWORMS: Staining of, Rukhadze and Blajin. J. Trop. Med. & Hyg. 32: 342, 1929.

Unfixed small trematodes and cestodes are stained for thirty to sixty minutes in a 30 per cent lactic acid solution in which 0.3 per cent of carmine has been dissolved at the boiling point; staining is checked by the microscope. Wash then in running water till purple; dehydrate as usual in alcohol and bring into Canada balsam. For large cestodes macerate in water for three to four days (in summer not more than twenty-four hours); stain for four to six hours; wash; immerse for sixteen to twenty hours in iron-carbolic (liq. ferri sesquichlor. 3 drops, carbolic acid 1 per cent 2 drops, water 100 c.c.); wash, straighten on a slide; dry at 30° C. to 37° C.; mount in Canada balsam or in resin dissolved in a mixture of chloroform and absolute alcohol or carbon disulphide.

OVA: Preservation of, Sankin, S. L. Arch. f. Schiffs- u. Trop-Hyg. 33: 416, 1929.

The feces are worked up with a Telemann solution containing only 50 per cent of hydrochloric acid, a concentrated solution deforming eggs. Of the following 10 c.c. is added:

Formalin 40 per cent	3
Common salt	0.85
Distilled water	100

The whole is centrifuged for a minute. The supernatant fluid is poured off, the precipitate washed with more of the formalin solution and precipitated again by the centrifuge. A drop of the sediment is placed on a clean slide, covered with a clean cover glass, and ringed with Canada balsam preferably liquefied by heat and not by xylol. Such preparations are claimed to keep well for months or years, and to show up well the eggs of nematodes, cestodes and trematodes.

BLOOD: New Peroxidase Stain, Brice, A. T., Jr. Stain Tech. 4: 100, 1930.

Oxydase Reagent: Dissolve 0.3 gm. of benzedine base in 100 c.c. of 95 per cent ethyl alcohol. Add 1 c.c. of a saturated aqueous solution of sodium nitroprusside. This reagent should keep equally as well as the Beacom modification of Good pasteur's stain, namely eight months to a year.

Hydrogen Peroxide: 1/200 solution made by adding 2 to 3 drops to 15 c.c. of distilled water. This must be freshly prepared immediately before use.

Wright's Stain according to the usual formula.

Thin films should be made and dried in air. The slide or cover slip is first flooded with the oxydase reagent which is allowed to act for from one to one and one-half minutes. An approximately equal portion of the hydrogen peroxide solution is then poured on the slide and allowed to act for three minutes. The slide is then washed thoroughly under the tap and dried by blotter and in air. The drying is necessary in order to remove the water from the film to permit of its being stained by an alcoholic stain.

The film is then stained by Wright's method according to the usual technic and employing normal time factors. The resultant stain with normal blood gives a very clear-cut picture of the differential characteristics of the cells.

As in using albumen, the slide must be clean before mounting the ribbon upon it. Place a small drop of gelatin fixative on the slide and smear with the finger so that only a thin film is present. Flood with a 2 per cent solution of formalin in distilled water. Mount the ribbon on the slide and warm slightly on a copper plate to straighten it. Then pour off the excess formalin solution, and allow the slide to dry for at least 12 hours at room temperature, or place in an oven at 30°. In working with material which has a tendency to become loose from the slide, place a small uncovered dish of concentrated formalin in the oven with the slides. The formalin renders the gelatin insoluble and thus holds the sections to the slide.

TISSUE: Rapid Stain for, Geschickter, C. F. Stain Tech. 5: 81, 1930.

To 4 parts of 1 per cent aqueous azure A (axure I) which has been previously filtered, add very rapidly one part of filtered 0.5 per cent aqueous Erie garnet B. The mixture is immediately filtered to prevent precipitation. Occasional refiltering may be necessary if the mixture has been standing a month or more. Float tissue slices from a freezing microtome directly into distilled water; pick them up with a round glass rod and immerse into the stain which has been poured into a small section dish. The staining time varies with different tissues but ten to fifteen seconds usually gives the most satisfactory results. Lift sections from the stain, still on the same glass rod, float through two changes of distilled water, and then transfer to a clean glass slide. Place a large drop of 40 per cent glucose on a clean cover slip and immediately invert over the section. The mounted specimen is then ready for microscopic examination. In examining the tissues under the microscope it is best to employ an intense artificial light (an ordinary 40 or 60 watt bulb is sufficient). After mounting, one-half to one minute should elapse before examining the section to allow it to clear.

STAIN: Flagella and Capsule, Bailey, H. D. Prac. Exper. Biol. & Med. 27: 111, 1929.

1. A small amount of material from a fifteen to twenty-four hour agar growth is diluted through two successive drops of water on a slide and a loopful of the second is smeared on a clean slide. Dry without heat.

2. Treat with mordant (5 per cent tannic acid 3 parts, 10 per cent $FeCl_3$ 1 part, preferably filtered before use) two minutes. Mordant will keep indefinitely.

3. Mix 7 drops of mordant with 1 drop Ziehl-Neelson carbolfuchsin. Add 1 drop concentrated HCl and mix. Add one drop formaldehyde (40 per cent?) and mix. Cover smear with this mixture freshly prepared after pouring off mordant; allow to stand seven minutes.

4. Wash in running water.

5. Apply Ziehl-Neelson carbolfuchsin and steam gently thirty seconds. Staining fluid may be a mixture of this with anilin gentian violet if more intense staining is desired. Stain should preferably be filtered before use.

6. Wash in running water.

Method may be used for staining exudate from white mice killed with pneumococci as follows: (1) Mix loopful of exudate with loopful of water and make a thin smear. (2) Apply mordant ten seconds. (3) Wash. (4) Apply cold diluted carbolfuchsin ten seconds. (5) Wash, blot, and examine.

AMEBA: Preservation of, Cherefeddin, O. Deutsche. med. Wchnschr. 55: 1084, 1929.

The longer stools are kept before examination, the greater the difficulty in finding and identifying ameba in them, an unavoidable disadvantage where samples of stools have to be sent to distant laboratories for examination. The author has found Bouin solution an excellent preservative. It consists of formalin 40 per cent, 10 gm.; acetic acid 2 gm.; aq. dist. 30 gm., picric acid to saturation. One-half to 1 c.c. of this solution is well mixed with 3 to 5 c.c. of stool, preferably the blood and mucus in the stool; the container, or test

tube, can be closed by rubber or ordinary cork. In this mixture the ameba retain their form for over ten days; endo- and ectoplasm, and nuclear structure remain distinct; furthermore, they can be stained with iron hematoxylin.

AMEBA: Culture of, Tanabe, M., and Chiba, E. Tokyo Med. News p. 2586, 1928.

The medium slant consists of mixture of Ringer's solution (NaCl 9.0, $CaCl_2$ 0.2, KCl 0.2, Aq. dist. 1000); agar-agar 10 gm.; asparagin 1.0 gm. Ringer's solution and 5 per cent rabbit's serum is added, just up to the top of the slant. Immediately before inoculation the medium is sterilized at 160 to 180° C. for one hour; and 2 or 3 platinum-loopfuls of rice powder are added. The medium is than warmed in the incubator at 37° C., inoculated with platinum loopfuls of infected feces, and cultivated at 37° C. In this medium the ameba live and proliferate vigorously for twelve to fourteen days. The P_H of the medium at the beginning is 7.8, but with proliferation of ameba it becomes 6.4. Subinoculations proved successful.

PERTUSSIS: Diagnosis by Cough Plate Method, Sauer, L. W., and Hambrecht, L. J. A. M. A. **95**: 263, 1930.

The medium used for the cough plate is easily made, as filtration and titration are unnecessary. To 500 gm. of peeled, sliced potatoes 40 c.c. of glycerin and 1000 c.c. of distilled water are added. This is boiled in a covered kettle until the potatoes are soft; the amount of water lost by evaporation is replaced by adjustment to the original weight, and the mixture is strained through gauze. To 500 c.c. of this filtrate, 1500 c.c. of 0.6 per cent salt solution and 60 gm. of agar are added. (This will make 2 liters of 3 per cent agar.) An asbestos mat and occasional stirring will prevent scorching. It is not necessary that all the agar be dissolved. Adjustment is made to the original weight and the mixture is bottled in 150 c.c. amounts and autoclaved. The agar can be stored in a refrigerator for several months. The sediment of potato débris that appears need cause no concern, as the melted agar can readily be poured off when the medium is used. The melted contents of a bottle is added to 30 c.c. or more of defibrinated blood, and plates are poured so that each petri dish contains about 20 c.c. of medium.

The agar should be allowed to cool to a few degrees (45° C.) above the solidifying point before it is added to the warmed blood; if too hot, it will darken the medium and render it unfavorable for the recognition of the characteristic pertussis colonies. It is advisable to make up the plates when needed and not to use them if they are more than a week old, as the surface of the medium soon dries out. Frozen plates cannot be used.

Plates should be incubated in an inverted position within a few hours after exposure. A few drops of water placed at the center of the cover is helpful if the surface of the medium appears dry. Colonies which spread rapidly, such as B. subtilis, B. mycoides and molds, should be cut out as soon as detected. Their presence can to a great extent be avoided by cleaning the table with a wet cloth just before the plates are poured. Plates exposed relatively late in the disease are seldom positive. A poorly exposed plate should not be incubated.

The incubated plate should be examined for colonies of B. pertussis at the end of the second day. If the colonies are very numerous, they may be seen as minute, glistening, mercury-like droplets amid the larger saprophytic colonies. By the end of the third or fourth day, as a rule, they will have more than doubled in size. The colony is translucent, markedly raised and definitely circular. In size, the average well-developed colony approximates 1 mm. in diameter. When viewed in reflected light a definitely darkened zone is seen to surround the whitish colony; in transmitted light this area including the colony appears distinctly lighter than the surrounding medium. This is characteristic of B. pertussis. The width of this zone may exceed twice the diameter of the colony. Plates which have turned very dark do not reveal these features. If the colonies are few, they may not be recognized until the fourth or even the fifth day.

STREPTOCOCCUS EPIDEMICUS: Isolation of, Pilot, I., Hallman, B., and Davis, D. J. J. A. M. A. 95: 265, 1930.

Ascites fluid, specific gravity from 1.010 to 1.016, from five different patients, gave uniform results. Horse serum may be used but is not as satisfactory. Defibrinated sheep's or horse's blood may be substituted for human blood. The minimum proportion of ascites fluid that will bring out the large colonies is 1 c.c. of the fluid to 10 c.c. of agar.

On the ascites blood agar in poured plates, Streptococcus epidemicus forms in the depths as elliptic or thin lens-shaped colonies surrounded by a clear zone of hemolysis from 4 to 8 mm. in diameter. A more opaque portion or nucleus appears in some part of the colonies on or near the surface or bottom of the plate. On the surface the colonies become very large, from 3 to 8 mm. in diameter, at first moist, transparent and biconvex, then flat, more opaque and gray, becoming wrinkled and dry in the center. The zone of hemolysis of the surface colonies is narrow, often being not more than 1 mm. Some hemolysis can be seen in the blood agar immediately under the colony. On the bottom of the plate the colonies spread out and form a large gray colony, homogeneous except for a small opaque nucleus. In deep tubes of ascites agar, similar elliptic smooth colonies appear in the interior of the medium; on the sides of the tube the colonies spread out to form large thin gray colonies.

On the same medium ordinary hemolytic streptococci, streptococci from scarlet fever and erysipelas form small biconvex colonies that may become flat. The colonies may occasionally spread slightly on the bottom of the plate but are not as large and usually do not present the more opaque or nuclear portions that are observed in Streptococcus epidemicus. The hemolytic zones appear uniform throughout the medium.

CULTURE MEDIA: Simple Method for Indicating Carbohydrate Series, Stone, R. V. Am. J. Pub. Health 20: 1140, 1930.

The author uses colored glass beads placed in the media tubes, each color indicating a different sugar. The beads are known as "No. 2 cut beads" and the most useful colors are: red (dextrose); opal (maltose); amber (mannite); blue (lactose); black (saccharose); and dark green (plain broth).

TISSUE: Rapid Staining Method for Spinal Cord and Stem, Knower, H. McE. Science 72: 172, 1930.

The method may be used on fresh or fixed material and after any fixative.

Cut, free hand, with a sharp, flat, thin razor, sections preferably 1 mm. or more thick, or frozen sections 100 micron thick.

From 95 per cent alcohol (minimum five minutes) transfer to Cole's mordant (50 per cent alcohol, 20 c.c.; ferric chloride 1 gm.; glacial acetic acid 2 c.c.) for at least five minutes, and then to alcohol. After prolonged mordanting staining is sometimes superfluous; but the following stain is recommended:

To 5 drops Cole's "stock hematoxylin" (absolute alcohol 20 c.c.; sodium bisulphite 0.2 gm.; water [distilled] 5 drops; light colored hematoxylin crystals 1 gm.) add 10 drops water and 1 drop ammonium hydroxide. After thirty seconds add 5 c.c. 95 per cent alcohol. Stain sections about five minutes and differentiate with 0.4 per cent HCl. If necessary, remove sediment with cotton or lens paper. Rinse in slightly alkaline 95 per cent alcohol after securing proper definition.

Study by strong reflected light.

FLAGELLA STAIN: Baily, H. D. Science 72: 95, 1930.

Make a thin smear from a fifteen to twenty-four hour agar growth, onto a clean slide, preferably transferred first to a drop of water and allowed to stand therein five minutes. Air-dry, without heat.

Cover with mordant (5 per cent tannic acid, 3 parts; 10 per cent ferric chloride, 1 part) two minutes. This solution will keep indefinitely.

Pour off mordant and cover with following mixture freshly prepared: 7 drops mordant mixed with 1 drop Ziehl-Neelson carbol fuchsin; 1 drop conc. HCl added and mixed; then 1 drop concentrated formaldehyde. Apply seven minutes, wash in running water.

Cover with Ziehl-Neelson carbol fuchsin (basic fuchsin 10 gm.; 95 per cent ethyl alcohol, 100 c.c.; 5 per cent aqu. phenol, 1000 c.c.) and steam gently one-half minute. Wash in running water. Blot.

In staining peritoneal exudate to demonstrate capsules of pneumococci, employ the following technic:

Spread loopful on glass slide either undiluted or mixed with a loopful of water.

Apply mordant for ten seconds. Wash in running water.

Apply cold diluted carbol fuchsin stain ten seconds. Wash with water; and blot.

SPIROCHETA PALLIDA: Improved Warthin-Starry Method for Single Sections, Farrier, R., and Warthin, A. S. Am. J. Syph. 14: 394, 1930.

Warthin-Starry Method as Modified to Date:

1. Fix tissues in formol, imbed in paraffin, and make cover-slip preparations.

2. Dry preparations in the oven for one to several hours then pass through flame, run it through two xylols, then two absolute ethyl alcohols, then 96 per cent ethyl alcohol, then water.

3. By means of a set of colorimetric standards using bromcresol green as an indicator, prepare a liter of water of P_H 4.4. This can be done approximately by adding fifteen drops of 1 per cent pure citric acid to a liter of distilled water. Shake thoroughly and let stand a few minutes before testing. Adjust to P_H 4.4 by adding more acid or more water.

4. Prepare a 2 per cent silver nitrate solution from this adjusted water.

5. From a part of this prepare a 0.5 per cent to 1 per cent solution of silver nitrate with the adjusted water.

6. Place enough of this 0.5 per cent to 1 per cent silver nitrate in a perfectly clean bottle so that when the cover slip is placed in it on edge the solution will cover the section by capillary attraction.

7. Rinse the preparation in the adjusted water and place in the silver solution with the section side facing the side of the bottle. Impregnate in a dark place at 37.5° C. for thirty minutes.

8. Prepare a 5 per cent gelatin and a 5 per cent hydroquinone solution. Keep the gelatin at about 45° C.

9. When section is ready to reduce, place the cover slip section-side up in a small flat dish.

10. Add 15 c.c. of the warm 5 per cent gelatin to 3 c.c. of 2 per cent silver nitrate. Mix and add 1 c.c. of 5 per cent hydroquinone. Mix rapidly and pour over the section.

11. Reduce until the gelatin begins to turn brownish-black.

12. Rinse in warm distilled water.

13. Place in 5 per cent sodium hyposulphite for a few minutes.

14. Wash in distilled water, then 96 per cent ethyl alcohol, then two changes of absolute ethyl alcohol, then two changes of xylol, and finally mount in balsam.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Pathology of Diabetes Mellitus**

ALTHOUGH diabetes is one of the oldest of recognized diseases, its etiology is still a matter of dispute and conjecture. The prolongation of life now possible with insulin treatment, and the growing importance of the complications of the disease render this survey of its pathology both timely and warranted.

The material upon which this book is based—300 autopsied cases—and the ability of the author render it a useful and valuable contribution to a complicated and, as yet, little understood subject.

After a succinct yet thorough résumé of the normal pancreas, thirty-one pages are given to a discussion of the pancreatic changes seen in diabetes, including the apparently normal glands which are encountered in a definite proportion of cases (27 per cent of this series) to the bewilderment of pathologist and clinician.

Three hypotheses are suggested as a possible explanation. (1) The islands may be normal and insulin produced, but are either defective in quality or neutralized by some substance in the body; (2) The diabetes may be other than insular in origin; (3) The insulin may be formed normally but through failure in transport is not distributed where it is needed.

A large proportion of the book, and a very valuable section repaying careful perusal, is devoted to the pathologic evidences of abnormal carbohydrate and fat mechanism, following which are chapters on the pathology of acidosis and coma; arteriosclerosis and gangrene; the heart; diabetes and infection; pathology of diabetes in children; pathology of diabetes in longstanding cases; hemochromatosis; insulin-resistant cases; hyperinsulinism and pathologic effects of insulin administration; endocrine disturbances; and a final chapter on theories as to the etiology of diabetes.

An appendix on methods used in the investigation is somewhat brief, and in a further edition could well be expanded to give the methods referred to in detail.

The book is profusely illustrated, the illustrations being well chosen and excellently reproduced.

The volume can be accepted as a valuable contribution to the study of a prevalent and puzzling disease.

*The Pathology of Diabetes Mellitus. By Shields Warren, M.D., Pathologist to the New England Deaconess Hospital, The New England Baptist Hospital and the Huntington Memorial Hospital, Boston; Director of Massachusetts State Tumor Diagnosis Service; Instructor in Pathology at Harvard Medical School, Boston, Mass. 212 pages, 83 engravings, 2 colored plates, cloth. Lea & Febiger, Phila., Pa.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

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EDITORIALS

Recent Studies on Asiatic Cholera

THE importance of Asiatic cholera as an epidemic disease has led to innumerable studies of the disease and its cause, the cholera vibrio first described by Koch in 1883. Despite the extensive investigations which have been carried out we are still ignorant of the factors which regulate the spread of an epidemic and lead to its arrest.

The recent report of d'Herelle, Malone, and Lahiri* is of interest, therefore, as approaching the problem from a different angle and advancing for its solution new concepts.

The authors initiate their report with the comment that, hitherto, studies upon cholera have been largely based upon laboratory animal inoculation; that the disease thus produced in the guinea pig is factitious, a laboratory artefact, as it were; and that an understanding of the pathology of the disease can come only from its study as it occurs in man from the beginning to the end.

*D'Herelle, F., Malone, R. H., and Lahiri, M. U.: Studies on Asiatic Cholera, Indian J. Med. Res. (Memoir 14), 1930.

Such a study they have attempted to carry out involving not only bacteriologic investigations but also a study of the disease as it occurred in villages of the Punjab of 1000 to 3000 inhabitants.

As a result they present, as worthy of consideration, certain concepts based upon their work.

Cholera is frequently a water-borne disease but studies of these workers upon the vitality of the vibrio in water lead to the somewhat paradoxical conclusion that the cholera vibrio can remain alive a long time in the waters of those regions where cholera occurs only accidentally in the form of widely separated epidemics, but dies in a few hours in the waters of India where cholera is either endemic or occurs in the form of annual epidemics, these facts having no relation to the disinfection of water by the methods usually employed.

The explanation advanced by the present investigators will be noted below.

One of the most obscure phases in the problem of cholera is its pathology: how does the vibrio act and how does it multiply and die in the human body?

D'Herelle and his coworkers believe that variations in virulence are of essential importance and emphasize that the symptoms of cholera appear only when the vibrios have invaded and begun to multiply within the intestinal mucosa and that it is here and here only that the elaboration of toxins ("virulent enzymes") takes place, coupled with the production, also, of intoxication resulting from hemolysis.

They state, therefore, that, "Cholera is a syndrome which can be produced by any bacterium capable of (1) attacking the lining cells of the tissues which comprise the intestinal wall and at the same time capable of utilizing the cleavage products for its development and multiplication within the tissues themselves, and (2) secreting enzymes which are able to hemolyze the red blood cells of man."

The cholera vibrio of Koch specifically possesses this double property.

The phenomenon of recovery from cholera permits of two hypotheses: (1) Recovery may be *active*, in consequence of the reaction of the patient and the acquisition of immunity; or (2) *passive*, by reason of the destruction of the vibrios by an antagonistic principle foreign to the body itself.

While the first hypothesis has usually been accepted, d'Herelle and his associates recall that it is not in accord with the fact that relapses occur and that all the known facts suggest that immunity is the consequence and not the cause of recovery. Furthermore, the history of all cholera epidemics shows that the more prolonged the epidemic the greater the number of recoveries. They support and believe, therefore, the second hypothesis which leads, naturally, to the assumption that the antagonist of the vibrio must be present in the intestinal tract of those who recover. That this is so they set themselves to prove, and advance evidence to show that this antagonist is the cholera bacteriophage.

Extensive experiments are reported in support of this contention and well repay study in the original.

The authors are convinced from their studies that the cessation of a cholera epidemic is due to the diffusion from convalescents of cholera bacteriophages and that it is possible to reproduce this process experimentally by sowing selected cultures of the bacteriophages in the wells of infected villages, and suggest that, to overcome the native objection to the addition of anything to their drinking water, the bacteriophage culture might be isolated from the sacred Ganges River, the addition of which to well water might be favorably received.

They also report the therapeutic application of the bacteriophage per os and *never* by subcutaneous injection, with extremely favorable results.

The work thus reported covers long, extensive and careful experimentation and deserves the equally careful consideration of all those concerned with the prevention, management, and control of this extremely virulent infection.

—*R. A. K.*

The "Shift to the Left" and Nuclear Index of Polymorphonuclear Leucocytes

ANY methods tending to improve the practical diagnostic value of total and differential leucocyte counts is to be welcomed since these examinations probably rank next in frequency to routine urinalyses.

As a general rule the total leucocytes per cubic millimeter of blood and the percentage of polymorphonuclears constitute the chief information and yet these data may create erroneous impressions of the degree and progress of infection. As pointed out by Boerner[1] a more accurate and useful method is to calculate and express the "absolute" numbers of the different leucocytes, as one or more types may thereby show important changes which are not revealed by the usual percentage or "relative" system. This means, however, the task of forgetting percentages and training the mind to interpret results on the basis of the total numbers of the different leucocytes per cubic millimeter of blood.

Of even greater importance and practical value, however, are methods of counting the immature polymorphonuclear leucocytes as an index of the degree and progress of infection, the work of Arneth, Schilling and others being noteworthy in this field. In other words a differentiation or "splitting" as it were of the polymorphonuclears into mature and immature forms (called metamyelocytes) represents a distinct advance in differential leucocyte counting since the numbers of metamyelocytes is a better index of the degree and progress of infection than a "lump" percentage of these cells under the designation of polymorphonuclears. The proportion of these immature to mature cells is nicely expressed by the "nuclear index" described by Gerard and Boerner.[2, 3] Of course it varies according to age, the index for children under four years of age being from 2.81 to 4.48 while it is 15 or over for adults.

As shown by these investigators the index is determined by dividing the percentage of metamyelocytes (immature polymorphonuclears) into the percentage of mature cells. For example, if a differential count showed 4 per

cent metamyelocytes and 66 per cent polymorphonuclears the calculation would be made as follows:

$$66 - 4 = 62$$

$$\frac{62}{4} = 15.5 \text{ the normal index for adults.}$$

An index between 10 and 15 means a slight increase of metamyelocytes (indicative of infection) or a slight "shift to the left"; an index of 5 to 10 is a moderate "shift" while an index below 5 is a marked "shift." Thus in a glance one obtains the proportion of immature to mature cells and far more valuable information of the degree of infection than obtained from the percentage of polymorphonuclears expressed in the usual method of counting.

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—*J. A. K.*